

# THE PREDICTIVE EFFECT OF NEUTROPHIL AND PLATELET LYMPHOCYTE RATIOS ON MICROSCOPIC SENTINEL LYMPH NODE METASTASES IN STAGE 2 MALIGNANT MELANOMA PATIENTS\*

## KLİNİK OLARAK EVRE 2 MALİGN MELANOM HASTALARINDA NÖTROFİL VE TROMBOSİT LENFOSİT ORANLARININ SENTİNEL LENF NODU MİKROSKOPİK METASTAZI HAKKINDAKİ ÖNGÖRÜ ETKİLERİ

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\*The study has been presented as an oral presentation during the 44th National Meeting of the Turkish Society of Plastic Reconstructive and Aesthetic Surgery in Antalya, on November 2022.

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**Citation/Atf:** Kozanoğlu E, Akalin BE, Aksöyler DY, Berköz HÖ, Jahangirov A, Kumbaşı T, et al. The predictive effect of neutrophil and platelet lymphocyte ratios on microscopic sentinel lymph node metastases in stage 2 malignant melanoma patients. Journal of Advanced Research in Health Sciences 2024;7(2):68-72. <https://doi.org/10.26650/JARHS2024-1417909>

### ABSTRACT

**Objective:** Malignant melanoma is a life-threatening disease, and the stage affects both treatment and survival. Staging is based on the microscopic Breslow thickness of the tumor, ulceration, and lymph node status. This study compares the neutrophil and platelet lymphocyte ratios of patients with and without micrometastatic sentinel lymph nodes and assesses the predictive value of these ratios.

**Materials and Methods:** The study includes patients who had a sentinel lymph node biopsy between January 2017 and December 2021 that resulted in a T value of T2b or T3a with neither any regional lymph node metastasis (N0) nor any systemic metastasis (M0). These patients were diagnosed with Stage 2A melanoma. The study evaluates the relationships patients' sentinel lymph node status and gender have with their neutrophil and platelet lymphocyte ratios.

**Results:** The study includes 65 patients, 24 of who are female and 41 of whom are male. The mean age is 55.58 years (Range = 18 to 87). The neutrophil, lymphocyte, and platelet counts and neutrophil and platelet lymphocyte ratio (NLR and PLR) did not significantly differ between the patients with and those without micrometastasis ( $p>0.05$ ). The difference showed no significance whether adjusting for age and gender. A significant difference was found between the patients with and those without micrometastasis with respect to gender ( $p=0.009$ ), with the percentage of males being significantly higher in the patients with micrometastasis.

**Conclusion:** Neutrophil and platelet lymphocyte ratios have not been found to be independent predictors of sentinel lymph node micrometastasis in Stage 2A malignant melanoma, with male patients in this stage having a higher risk of sentinel lymph node micrometastasis.

**Keywords:** malignant melanoma, micrometastasis, neutrophil lymphocyte ratio, platelet lymphocyte ratio, sentinel lymph node biopsy

### ÖZ

**Amaç:** Yaşamı tehdit eden bir deri kanseri olan malign melanomda hastalığın evresi tedavi yaklaşımını ve sağkalımı etkilemektedir. Malign melanomun evrelemesinde, tümörün mikroskopik olarak ölçülen Breslow kalınlığından, ülserasyondan ve olası bir lenf nodu tutulumundan faydalanılmaktadır. Bu çalışmada sentinel lenf nodu biyopsileri mikroskopik olarak metastazsız (negatif) ve metastazlı (pozitif) olan hastaların nötrofil lenfosit ve trombosit lenfosit oranları karşılaştırıldı ve bu oranların metastaz üzerinde bir öngörü etkisi olup olmadığı değerlendirildi.

**Gereç ve Yöntem:** Ocak 2017–Aralık 2021 tarihleri arasında sentinel lenf nodu biyopsisi ameliyatı yapılmış olan, Breslow kalınlığına göre T değeri T2b veya T3a olan, makroskopik lenf nodu metastazı olmayan (N0) ve sistemik (uzak) metastazı da olmayan (M0) evre 2A malign melanom hastaları çalışmaya dahil edildi. Sentinel lenf nodu biyopsisi durumları ile cinsiyet arasındaki ilişki ve sentinel lenf nodu biyopsisi ile nötrofil ve trombosit lenfosit oranları arasındaki ilişki değerlendirildi.

**Bulgular:** Çalışmaya 65 hasta dahil edildi. Hastaların 24'ü kadın, 41'i erkekti. Hastaların ortalama yaşı 55,58 idi (18–87). Yaş ve cinsiyete göre düzeltme yapmadan ve yapılarak gerçekleştirilen değerlendirmeler sonucunda mikrometastaz açısından nötrofil, lenfosit, platelet sayıları, nötrofil lenfosit oranı (NLO) ve platelet lenfosit oranları (PLO) değerleri bakımından istatistiksel olarak anlamlı fark saptanmadı ( $p>0,05$ ). Sentinel lenf nodu biyopsisi mikrometastazı olan ve olmayan hastalar arasında cinsiyet bakımından istatistiksel olarak anlamlı fark olduğu saptandı ( $p=0,009$ ). Mikrometastazlı grupta erkek yüzdesinin mikrometastazsız gruptan daha yüksek olduğu saptandı.

**Sonuç:** Evre 2A malign melanom hastalarında, nötrofil ve trombosit lenfosit oranları, sentinel lenf nodundaki mikroskopik metastazı bağımsız bir değişken olarak öngörememektedir. Bu evredeki erkek hastalarda sentinel lenf nodu mikrometastazı riski kadın hastalara göre daha yüksektir.

**Anahtar Kelimeler:** Malign melanoma, mikrometastaz, nötrofil lenfosit oranı, sentinel lenf nodu biyopsisi, trombosit lenfosit oranı

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Submitted/Başvuru: 11.01.2024 • Revision Requested/Revizyon Talebi: 23.01.2024 • Last Revision Received/Son Revizyon: 04.02.2024

• Accepted/Kabul: 05.02.2024 • Published Online/Online Yayın: 27.02.2024



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## INTRODUCTION

Malignant melanoma of the skin may be a life-threatening cancer, and the stage of the disease affects both the treatment and survival (1). With the introduction of new treatment options, the 5-year survival rate may exceed 95% in Stage 1 patients, whereas it may be as low as 10% in Stage 4 patients (1). Thus, early diagnosis and precise therapy are mandatory before the regional and systemic spread of the disease (2).

The microscopic Breslow thickness of the primary tumor, the presence of ulceration, and lymph node metastasis status are utilized when staging malignant melanoma (3). The risk of lymph node metastasis increases with greater Breslow thickness and the presence of ulceration (1). During the routine work-up with physical examination and imaging studies, the patients may not have experienced any macroscopic lymph node spread; however, thick and ulcerated melanomas are prone to microscopic metastases that may be detected with sentinel lymph node biopsies (4, 5). This method enables the pathological staging of the patient, from which additional surgeries and oncological treatments may be planned (4, 5).

Neutrophil and platelet lymphocyte ratios (NLR and PLR) are used in order to evaluate patients' immune status and can be calculated from peripheral blood cell counts (6). Wade et al. suggested utilizing these ratios in order to predict the sentinel lymph node spread of malignant melanoma (6). In addition to tumor thickness and the presence of ulceration, NLR and PLR have been demonstrated to be able to predict lymph node metastasis in malignant melanoma (7). The aim of this study is to evaluate the sentinel lymph node biopsies of patients with T2b and T3a tumors based on Breslow thickness who have no clinical lymph node metastasis (N0) nor any systemic metastasis (M0). The study assesses the absence or presence of microscopic nodal disease by comparing the NLR and PLR and investigates the predictive effect these ratios have regarding micrometastasis.

## MATERIALS and METHODS

The study was approved by the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 22.03.2022, No: 06). Informed consent was obtained from each patient.

The study has a retrospective design and utilizes institutional digital health records. This study includes patients with T2b and T3a malignant melanoma who have neither any macroscopic lymphatic (N0) nor systemic (M0) metastases, who are classified as having stage 2A malignant melanoma, and who then have had sentinel lymph node biopsies. Patients over 18 years of age were included without any limitation in terms of gender. The study period occurred between January 2017-December 2021 to ensure that the same surgical team performed the operations and monitoring throughout the process, thereby minimizing any procedural or technical treatment variations.

This study includes patients who had had peripheral blood cell

counts two weeks prior to surgery and had no overt clinical or serological infectious disease during these counts in order to avoid infection-related changes to their neutrophil, platelet, and lymphocyte values. The study excluded patients whose operations and pathological evaluations had been performed at other institutions, as well as patients who'd had no sentinel lymph node biopsy. The study also excluded pediatric patients, patients with an overt clinical or serological infectious disease two weeks pre-surgery, and patients whose malignant melanoma stage was other than 2A.

The study recorded each patient's age; gender; absolute neutrophil, platelet, and lymphocyte counts; neutrophil and platelet lymphocyte ratios; and the absence or presence of microscopic sentinel lymph node metastasis. Sentinel lymph node biopsy status was assessed with regard to gender and neutrophil lymphocyte and platelet lymphocyte ratios.

## Statistical analysis

The study used the program R (version 2.15.3; R Core Team, 2013) for the statistical analyses, with minimums, maxima, means, standard deviations, medians, frequencies, and percentages being used to report the data. The normal distribution of the quantitative data was assessed with both the Shapiro-Wilk test and graphical evaluations. The two-group comparison of the normally distributed variables was performed with the independent groups t test. The Pearson chi-square test was used to compare the gender percentages. The groups were corrected for age and gender, and the analysis of covariance (ANCOVA) test was performed to evaluate the groups, with a  $p < 0.05$  being accepted as statistically significant.

## RESULTS

The study includes 65 patients, of who 24 are female and 41 are male. The patients' mean age is 55.58 years (Range=18–87). The blood count values, neutrophil and platelet lymphocyte ratios, and sentinel lymph node biopsy status are presented in Table 1. The ages of patients showed no statistically significant difference with regard to the presence or lack of sentinel lymph node micrometastasis ( $p > 0.05$ ).

Table 2 exhibits the comparison of the blood count values and neutrophil and platelet lymphocyte ratios with respect to the sentinel lymph node micrometastasis status. In addition, the data have been evaluated with respect to this status' dependence on gender. When not classifying the data according to age and gender, the sentinel lymph node micrometastasis status shows no statistically significant difference regarding absolute neutrophil, platelet, and lymphocyte values or NLR and PLR ( $p > 0.05$ ). When classifying the data according to age and gender, sentinel lymph node micrometastasis status still showed no statistically significant difference in terms of absolute neutrophil, platelet, and lymphocyte values or NLR and PLR ( $p > 0.05$ ).

Table 3 demonstrates the relationship between sentinel lymph node micrometastasis status and gender. A statistically signi-

**Table 1: Demographic data, peripheral blood count values ad sentinel lymph node status**

	Minimum – Maximum (Median)	Mean±Standard deviation
Age	18-87 (57)	55.58±16.45
Neutrophil	2.5-8.4 (4.8)	4.95±1.36
Lymphocyte	0.5-3.6 (2)	2.06±0.59
Platelet	159-507 (266)	265.26±64.73
Platelet lymphocyte ratio	59.16-476 (131.17)	140.87±62.43
Neutrophil lymphocyte ratio	1-15 (2.4)	2.77±1.96
	<b>n</b>	<b>%</b>
<b>Gender</b>		
Female	24	36.9
Male	41	63.1
<b>Sentinel lymph node biopsy</b>		
Negative	44	67.7
Positive	21	32.3

n: Number of Patients

**Table 2. The relationship between the blood count values and the sentinel lymph node status**

	Negative sentinel lymph node biopsy	Positive sentinel lymph node biopsy	p (independent of gender, tested with independent groups t test)	p (dependent on gender, tested with ANCOVA)
	Mean ± Standard Deviation	Mean ± Standard Deviation		
Neutrophil	4.83±1.34	5.21±1.42	0.285	0.518
Lymphocyte	2.08±0.62	2.03±0.51	0.750	0.953
Platelet	268.48±73.47	258.53±41.62	0.567	0.569
Platelet lymphocyte ratio	143.47±71.19	135.44±39.10	0.631	0.563
Neutrophil lymphocyte ratio	2.78±2.27	2.75±1.08	0.965	0.770

**Table 3. The relationship between sentinel lymph node micrometastasis status and gender demonstrated as number of patients and percentage**

	Negative sentinel lymph node biopsy	Positive sentinel lymph node biopsy	p value (Pearson chi – square test)
	Number of patients (Percentage)	Number of patients (Percentage)	
<b>Gender</b>			<b>0.009*</b>
Female	21 (47.7)	3 (14.3)	
Male	23 (52.3)	18 (85.7)	

ificant difference was found between patients with and those without sentinel lymph node micrometastasis with respect to gender (p=0.009), with a higher percentage of male patients in the group with micrometastasis, while no such difference was found in the group without micrometastasis.

## DISCUSSION

Although malignant melanoma is not the most common skin cancer, it has a higher mortality risk than other skin cancer

types (8). The survival rate has increased with the developments in diagnostic studies and oncological treatments (2). Similar to all types of cancer, stage is the main predictor of survival in malignant melanoma, with precise staging being mandatory during the initial diagnosis (3).

If regional and systemic metastases are not detected in the initial evaluation and imaging examinations, the primary lesion should be excised and sent for appropriate pathological

examination regarding such things as the measurement of the Breslow thickness and the assessment of the ulceration status (5, 8). A Breslow thickness greater than or equal to 1.0 millimeter and the presence of ulceration necessitate a sentinel lymph node biopsy in order to detect any possible microscopic metastasis (4, 8).

Many novel studies have been performed in order to find new and independent variables apart from the Breslow thickness and the presence of ulceration in order to detect any possible sentinel lymph node micrometastasis (1, 6, 7, 9-12). Such an independent variable may help predict the stage of the disease even prior to invasive procedures such as the excision of the primary lesion and a sentinel lymph node biopsy. Mancuso et al. found interleukin and interferon levels of the peripheral blood to help predict metastasis in early stage malignant melanoma (9). Neutrophil and platelet lymphocyte ratios of the peripheral blood have also been found to help predict the survival and response to oncological treatments for advanced stage malignant melanoma (11-14). This study has been hypothesized with respect to the current usage of NLR and PLR in malignant melanoma.

Robinson et al. evaluated 1,489 patients with malignant melanoma and found NLR to be able to help predict microscopic sentinel lymph node metastasis (7). In fact, they defined cut-off values for both microscopic and clinical metastases and evaluated patients in all stages (7). The present study included only Stage 2A malignant melanoma patients in order to avoid confounding factors such as the Breslow thickness and the presence of ulceration. The study has assessed the predictive value of NLR and PLR for local and regional metastases in the early stage and found these two factors to not be independent variables.

High NLR and PLR have been found to be inversely proportional to survival rate in many studies (11, 14). However, Wade et al. demonstrated NLR and PLR to have different patterns in early and advanced stage malignant melanoma (6), finding high NLR to enable the regression of the primary tumor in the early stage of the disease (6). Another study by Wade et al. screened patient databases and prepared a nomogram called MelRisk for detecting sentinel lymph node micrometastasis in malignant melanoma (10). Patient's age, Breslow thickness, the presence of ulceration, the anatomic localization of the primary tumor, and NLR were able to be added to this nomogram, allowing the probability of micrometastasis to be found as a percentage (10). They found the predictive effect regarding sentinel lymph node micrometastasis to be augmented upon NLR being included in the nomogram (10). The current study has been based on the findings from MelRisk and compared the NLR and PLR values for patients in the same early stage of malignant melanoma. However, the study did not find these variables to help predict sentinel lymph node micrometastasis in Stage 2A melanoma patients.

Several studies have found a relationship between the probability of sentinel lymph node micrometastasis and gender with regard to malignant melanoma (15-17). Scoggins et al. found the primary tumor to have worse pathological properties and higher metastasis potential in male patients (15). Joosse et al.

found local tumors to metastasize less and survival rates to be better in female patients (16). Mervic et al. showed similar results and found the survival rate to be better in the female patients with metastasis (17). A higher percentage of males occurred in this study in the group with micrometastasis, and this finding is in concordance with the literature.

The strengths of this study are that it includes patients who were at the same disease stage, who had the same surgeons perform their operations, who had the same pathologists evaluate the specimens, and who had the same nuclear medicine physicians mark the sentinel lymph nodes. Future studies may increase the number of the patients and/or separately evaluate the patients at other disease stages who have no clinical metastases in order to be able to assess the predictive effect of NLR and PLR on sentinel lymph node micrometastasis for different patient groups.

## CONCLUSION

NLR and PLR as independent variables failed to predict sentinel lymph node micrometastasis in Stage 2A malignant melanoma patients, and these variables should be reevaluated over a larger patient cohort. Also, male patients were found to have a higher risk of sentinel lymph node micrometastasis in this stage.

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**Ethics Committee Approval:** This study was approved by İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 22.03.2022, No: 06).

**Informed Consent:** Informed consent was obtained from each patient.

**Peer Review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study- E.K., B.E.A., D.Y.A., H.Ö.B., A.J., T.K., U.E., R.A.A.; Data Acquisition- E.K., B.E.A., D.Y.A., H.Ö.B., A.J., T.K., U.E., R.A.A.; Data Analysis/Interpretation- E.K., B.E.A., D.Y.A., H.Ö.B., A.J., T.K., U.E., R.A.A.; Drafting Manuscript- E.K., B.E.A., D.Y.A., H.Ö.B., A.J., T.K., U.E., R.A.A.; Critical Revision of Manuscript- E.K., B.E.A., D.Y.A., H.Ö.B., A.J., T.K., U.E., R.A.A.; Final Approval and Accountability- E.K., B.E.A., D.Y.A., H.Ö.B., A.J., T.K., U.E., R.A.A.; Material and Technical Support- E.K., B.E.A., D.Y.A., H.Ö.B., A.J., T.K., U.E., R.A.A.; Supervision- E.K., B.E.A., D.Y.A., H.Ö.B., A.J., T.K., U.E., R.A.A.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The author declared that this study has received no financial support.

## REFERENCES

1. Pinto-Paz ME, Cotrina-Concha JM, Benites-Zapata VA. Mortality in cutaneous malignant melanoma and its association with Neutrophil-to-Lymphocyte ratio. *Cancer Treat Res Commun*

- 2021;29:100464.
2. Knackstedt T, Knackstedt RW, Couto R, Gastman B. Malignant melanoma: diagnostic and management update. *Plast Reconstr Surg* 2018;142(2):202e-16e.
  3. Keung EZ, Gershenwald JE. The eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system: implications for melanoma treatment and care. *Expert Rev Anticancer Ther* 2018;18(8):775-84.
  4. El Sharouni M-A, Witkamp AJ, Sigurdsson V, van Diest PJ. Trends in sentinel lymph node biopsy enactment for cutaneous melanoma. *Ann Surg Oncol* 2019;26(5):1494-502.
  5. El Sharouni M-A, Witkamp AJ, Sigurdsson V, van Diest PJ. Probability of sentinel lymph node positivity in melanoma. *Eur J Cancer* 2019;116:10-2.
  6. Wade RG, Robinson AV, Lo MC, Keeble C, Marples M, Dewar DJ, et al. Baseline neutrophil-lymphocyte and platelet-lymphocyte ratios as biomarkers of survival in cutaneous melanoma: a multicenter cohort study. *Ann Surg Oncol* 2018;25(11):3341-9.
  7. Robinson AV, Keeble C, Lo MC, Thornton O, Peach H, Moncrieff MD, et al. The neutrophil-lymphocyte ratio and locoregional melanoma: a multicentre cohort study. *Cancer Immunol Immunother* 2020;69(4):559-68.
  8. Pavri SN, Clune J, Ariyan S, Narayan D. Malignant melanoma: beyond the basics. *Plast Reconstr Surg* 2016;138(2):330e-40e.
  9. Mancuso F, Lage S, Rasero J, Díaz-Ramón JL, Apraiz A, Pérez-Yarza G, et al. Serum markers improve current prediction of metastasis development in early-stage melanoma patients: a machine learning-based study. *Mol Oncol* 2020;14(8):1705-18.
  10. Wade RG, Bailey S, Robinson AV, Lo MC, Peach H, Moncrieff MD, et al. MelRisk: Using neutrophil-to-lymphocyte ratio to improve risk prediction models for metastatic cutaneous melanoma in the sentinel lymph node. *J Plast Reconstr Aesthet Surg* 2022;75(5):1653-60.
  11. Wang E, Huang H, Tang L, Tian L, Yang L, Wang S, et al. Prognostic significance of platelet lymphocyte ratio in patients with melanoma: A meta-analysis. *Medicine (Baltimore)* 2021;100(38):e27223.
  12. Pan M, Alavi M, Herrinton LJ. Association of inflammatory markers with disease progression in patients with metastatic melanoma treated with immune checkpoint inhibitors. *Perm J* 2018;22:17.
  13. Guida M, Bartolomeo N, Quaresmini D, Quaglino P, Madonna G, Pigozzo J, et al. Basal and one-month differed neutrophil, lymphocyte and platelet values and their ratios strongly predict the efficacy of checkpoint inhibitors immunotherapy in patients with advanced BRAF wild-type melanoma. *J Transl Med* 2022;20(1):1-15.
  14. Cohen JT, Miner TJ, Veziridis MP. Is the neutrophil-to-lymphocyte ratio a useful prognostic indicator in melanoma patients? *Melanoma Manag* 2020;7(3):MMT47.
  15. Scoggins CR, Ross MI, Reintgen DS, Noyes RD, Goydos JS, Beitsch PD, et al. Gender-related differences in outcome for melanoma patients. *Ann Surg* 2006;243(5):693.
  16. Joosse A, De Vries E, Eckel R, Nijsten T, Eggermont AM, Hölzel D, et al. Gender differences in melanoma survival: female patients have a decreased risk of metastasis. *J Investigative Dermatol* 2011;131(3):719-26.
  17. Mervic L. Time course and pattern of metastasis of cutaneous melanoma differ between men and women. *PloS One* 2012;7(3):e32955.