Research Article / Araştırma Makalesi

Malign Melanom Tanılı Hastalarda Klinikopatolojik Özellikler Ve Nötrofil-Lenfosit Oranının Prognostik ve Prediktif Önemi

Clinicopathological Features of Patients with Malignant Melanoma Diagnosis and Prognostic and Predictive Importance of Neuthrophil-Lymphocyte Ratio

[1]Yasemin Sağdıç Karateke, [2]Lütfiye Demir, [1]Murat Dinçer, [1]Bülent Yıldız

[1]Eskişehir Osmangazi Universty, Department of Medical Oncology, Faculty of Medicine, Eskişehir, Türkiye
[2]Biruni Universty, Medicana İnternational İzmir Hospital, Department of Medical Oncology, Izmir,Türkiye

Abstract: In this study, the effect of demographic, laboratory and clinicopathological parameters along with neutrophil-lymphocyte ratio (NLR) on prognosis and survival and correlation with other parameters was researched in patients with malignant melanoma (MM) diagnosis. In our study, 107 patients monitored for MM diagnosis in Eskişehir Osmangazi University Medical Oncology clinic from 2010-2017 were retrospectively assessed. Age, gender, LDH level, pathological parameters, BRAF mutation status, neutrophil-lymphocyte ratio (NLR) and the effects of these parameters on overall survival (OS) and disease-free survival (DFS) and correlations with each other were researched. At time of diagnosis, 86% of patients were in the early stage. The dominant types identified were cutaneous MM and nodular MM. Median NLR cut-off value was identified as 1.97. All non-cutaneous MM cases were BRAF negative (p<0.0001). High NLR was associated with advanced stage (p=0.001), advanced age (p=0.008), ulceration presence (p=0.011), and high mitosis count (p=0.05). High NLR (p<0.0001), high LDH level (p=0.04), increased Breslow thickness (p=0.01), increased Clark level (p=0.01), high mitosis count (p=0.02), and lymph node (LN) involvement (p=0.04) were correlated with significantly shorter OS durations. Cox multivariate regression analysis identified the most effective independent parameters on OS were LN involvement (HR: 3.4, p=0.01) and high NLR (HR: 4.6, p=0.04). Nodal involvement was also identified as the most predictive independent parameter for recurrence (HR: 3.2, p=0.03). In addition to classic parameters, NLR appears to be a biomarker which can predict prognosis. Patients with nodal involvement and high NLR values should be monitored more closely in clinics. Data require support with broad-scale studies.
Keywords: Malignant melanoma, Neutrophil-lymphocyte ratio, Prognostic factors

Özet: Bu çalışmada MM tanılı hastalarda demografik, labaratuvar özellikler ve klinikopatolojik parametrelerle birlikte nötrofil-lenfosit oranının (NLR) prognoz ve sağkalım üzerine etkisi ve diğer parametrelerle ilişkisi araştırıldı. Çalışmamızda 2010-2017 yılları arasında Eskişehir Osmangazi Üniversitesi Tıbbi Onkoloji Kliniği'nde takip edilen MM tanılı 107 hasta retrospektif olarak değerlendirildi. Yaş, cinsiyet, LDH seviyesi, patolojik parametreler, BRAF mutasyon durumu, nötrofil-lenfosit oranı(NLR) ile bu parametrelerin genel sağkalım(OS) ve hastalıksız sağkalım(DFS) üzerine etkisi ve birbirleri ile ilişkisi araştırıldı. Hastaların tanı anında %86'sı erken evredeydi. Kutanöz MM ve nodüler malign melanom (NMM), ağırlıklı saptanan tipti. Medyan NLR cut-off değeri 1.97 saptandı. Non-kutanöz MM olgularının hepsi BRAF negatifti (p<0.0001). Yüksek NLR ileri evre (p=0.001), ileri yaş (p=0.008), ülserasyon varlığı (p=0.011), yüksek mitoz sayısı (p=0.05) ile ilişkiliydi. Yüksek NLR (p<0.0001), yüksek LDH seviyesi (p=0.04), artmış Breslow kalınlığı (p=0.01), artmış Clark düzeyi (p=0.01), yüksek mitoz sayısı (p=0.02), LN (Lenf nodu) tutulumu (p=0.04) anlamlı olarak daha kısa OS süreleri ile ilişkiliydi. Cox çok değişkenli regresyon analizlerinde OS üzerinde en etkili bağımsız değişken parametreler LN tutulumu (HR:3.4 p=0.01) ve yüksek NLR (HR:4.6 p=0.04) olarak saptandı. Nodal tutulum ayrıca nüksü en çok predikte eden bağımsız parametre olarak saptandı (HR:3.2 p=0.03). Klasik parametreler yanında NLR de prognozu öngördürebilecek bir biyobelirteç gibi görünmektedir. Nodal tutulumu olan ve NLR değeri yüksek olan hastalar klinikte daha yakından takip edilmelidir. Verilerin geniş çaplı çalışmalarla desteklenmesine ihtiyaç vardır.
Anahtar Kelimeler: Malign melanom, Nötrofil-lenfosit oranı, Prognostik faktörler

ORCID ID of the authors: YSK. 0000-0002-3287-6248, LD. 0000-0003-0492-3454, MD. 0000-0002-9146-813X
BY. 0000-0002-9111-9132

Received 09.12.2022 ***Accepted*** 21.07.2023 ***Online published*** 31.07.2023

Correspondence: Yasemin SAĞDIÇ KARATEKE- Department of Medical Oncology, Faculty of Medicine, Eskişehir Osmangazi Universty, Eskişehir, Turkey e-mail: yasmins88@hotmail.com

Karateke Sagdic Y, Demir L, Dincer M, Yildiz B . Clinicopathological Features of Patients with Malignant Melanoma Diagnosis and Prognostic and Predictive Importance of Neuthrophil-Lymphocyte Ratio, Osmangazi Journal of Medicine, 2023;45(5): 651-664 ***Doi:*** *10.20515/otd.1226498*

1. Introduction

Malignant melanomas (MM) are malignant neoplasms derived from melanocytes and mostly derived from skin and are responsible for most skin cancer deaths in spite of representing less than 5% of all cutaneous malignancies. Melanocytes are generally found in the epidermis, rarely on mucosal surfaces and in uveal regions. Nearly 90% of melanoma cases are observed as cutaneous melanoma. Among cutaneous melanoma types, the most frequently observed are superficial spreading melanoma (SSM) with nodular malignant melanoma (NMM) in second place (1, 2).

There are a range of factors identified to affect prognosis in melanoma. These prognostic factors include Breslow thickness, presence of ulceration, mitosis count, lymph node (LN) involvement, satellite, microsatellite, in-transit metastasis presence, serum LDH level in metastatic stage, presence of cranial metastasis, advanced age, male sex, anatomic localization, Clark level, histologic subtype, lymphovascular invasion, correlation with nevus, tumor regression, mutation status, and amelanotic melanoma. Contrary to the antitumoral effects of the lymphoid system in melanoma pathogenesis, neutrophils have suppressive effects on T cell functions and are thought to play effective roles in tumor angiogenesis, invasion and metastasis (3). As a result, the new prognostic biomarker in recent times of high neutrophil-lymphocyte ratio (NLR) is predicted to be associated with poor prognosis and studies about NLR continue to increase.

Melanoma form as a result of mutations in the cell growth cycle providing additional functions to proto-oncogenes and mutations resulting in function loss of tumor-suppressing genes. The discovery of BRAF mutations on the mitogen activated protein (MAP) kinase signal pathway has provided significant contributions to advances in melanoma studies, and development of immunotherapy and new targeted treatment regimes.

In MM cases 40-60% are positive for BRAF mutations. According to the Cancer Genomic Atlas, RAS mutations are identified at rates of 25-30% (4). In situations when only systemic chemotherapeutic regimes were available, the overall survival for metastatic melanoma was much worse; however, treatment for metastatic melanoma entered a new period with the development of targeted agents and immunotherapy regimes. The 3-5-year survival with BRAF-MEK targeted treatments reached 40% (5, 6). Additionally, in MM which is known to be immunogenic, a close relationship was identified between the association of PDL-1 expression in tumor types like Renal Cell Cancer (RCC) with TIL (7). Immunotherapy regimes provide clear survival advantages for these tumor types, with 3-year survival rates for metastatic melanoma reaching 58% with these treatments (8, 9).

In this study, the demographic, clinicopathological and laboratory features of patients with MM diagnosis were assessed for effect on disease prognosis and correlation with each other, and findings were compared with literature data. Additionally, the aim was to assess the NLR value, predicted to be a new biomarker in recent years which has low cost, is very popular and is simply calculated in peripheral blood, for correlation with disease prognosis, correlation with other parameters and predictive effect.

2. Materials and Metods

This study retrospectively assessed 107 patients monitored with MM diagnosis from 2010-2017 in Eskişehir Osmangazi University Faculty of Medicine Medical Oncology clinic. The demographic characteristics of patients, macroscopic tumor type, localization, Breslow thickness, Clark level, presence of tumor ulceration, mitosis count, nodal status, stage at diagnosis, BRAF mutation status, absolute neutrophil, lymphocyte and LDH levels in peripheral blood, NLR value, recurrence and treatment for metastatic disease were determined. Taking hemograms at time of diagnosis as basis, the ratio of absolute neutrophil count to lymphocyte count (NLR) was calculated. Overall survival (OS) was defined as the duration from time of diagnosis to final meeting date or death, while disease-free survival (DFS) was defined as the

duration from operation date to when first local and/or distant recurrence was identified or the final meeting date for patients who did not attend follow-up.

2. Statistical Method

Statistical analysis of data used the "SPSS (Statistical Package for the Social Sciences) 25.0 for Windows" program. Descriptive statistics were used for initial analysis of the demographic, clinical and pathological features of patients. The Kaplan-Meier method was used for survival analyses, with log-rank regression analysis used to compare survival in groups. Comparison between A groups used the Pearson chi-square test, while Cox risk regression analysis was used to determine multiple independent variables effective on prognosis and survival. By calculating the mean of NLR values for all patients, the median NLR value was identified as 1.97. Additionally, the cut-off value for NLR was identified as 1.97 according to the ROC curve ($p<0.001$, area under the curve(AUC) 79.5%) (Figure 1). All patients were grouped as high and low according to NLR 1.97. Additionally, the cut-off value for LDH level at diagnosis for overall survival was identified as 297.5 U/L according to ROC curve ($p<0.0001$)

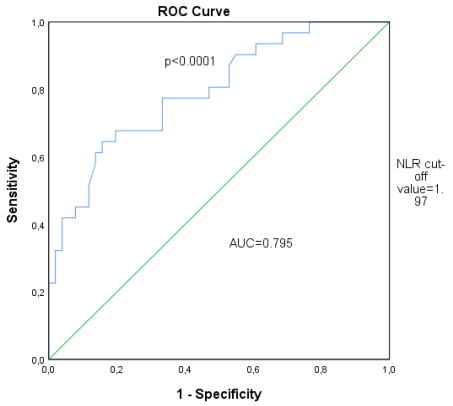

Figure 1. ROC curve used to determine cut-off values for NLR at time of diagnosis

3. Results

The mean age in our study was 55 years (19-87) with male and female numbers similar (n=55/n=52). Of patients, 80.4% (n=86) were identified to have cutaneous MM, with the most commonly observed subtype NMM (n=22). At time of diagnosis, 30.9% of patients (n=29) were stage 0-I, 28.7% were stage II (n=27), 24.5% (n=23) were stage III and 14% (n=15) were stage IV. Of patients with BRAF mutation examined (39/107), 53.8% were BRAF v600 positive (n=21). The frequency of metastasis regions in metastatic patients at diagnosis and later were lung metastasis (64.7%), distant LN metastasis (40.4%) and liver metastasis (39.2%). The demographic and clinical features, tumor pathological and clinical features are stated in general in the table (Table 1).

3.1. General Characteristics of Patients

Table 1. Clinical, demographic, molecular and pathological characteristics of patients

Variables	Number (n)	Percentage (%)
Gender		
Female	52	48.6
Male	55	51.4

Age		
<55	53	49.5
≥55	54	50.5
Tumor localization		
Uveal	13	12.1
Face-head, neck skin	33	30.8
Trunk	16	15
Upper extremity	14	13.1
Lower extremity	20	18.7
Mucosal region	8	7.5
Unknown	3	2.8
LDH value		
<297.5 U/L	35	44.3
≥297.5 U/L	44	55.7
NLR		
<1.97	41	50
≥1.97	41	50
Recurrence		
Present	41	45.1
Absent	50	54.9
Surgical margin		
Negative	75	84.3
Positive	14	15.7
Stage at time of diagnosis		
In situ-stage I	29	30.9
Stage II	27	28.7
Stage III	23	24.5
Stage IV	15	16
Tumor type		
Uveal MM	13	12.1
YYM	17	15.9
LMM	8	7.5
NMM	22	20.6
ALM	9	8.4
Spitzoid MM	1	0.9
Mucosal MM	8	7.5
Other cutaneous types	24	22.4
Unclassified cutaneous MM	5	4.7
Clark level		
Stage I	11	14.7
Stage II	13	17.3
Stage III	16	21.3
Stage IV	28	37.3
Stage V	7	9.3
Ulceration		
Present	31	47
Absent	35	53
Mitosis (mm^2)		

≤4	35	59.3
≥5	24	40.7
BRAF mutation		
Positive	21	59.3
Negative	18	40.7
Metastasis regions		
Lung	33	64.7
Distant lymph node	21	40.4
Liver	20	39.2
Cranial	19	37.3
Bone	15	28.8
Surrenal	10	19.6
Skin-soft tissue	10	19.6
Peritoneum	8	15.4
Mucosal	2	3.9

NLR: *neutrophil-lymphocyte ratio* ***IFN:*** *Interferon* ***MM:*** *Malignant melanoma* ***SSM:*** *superficial, spreading melanoma* ***LMM:*** *Lentigo malignant melanoma NMM: Nodular malignant melanoma,* ***ALM:*** *Acral lentiginous melanoma* ***IFN:****Interferon* ***LN:****lenf nodu*

BRAF Mutation Status and Correlation with Other Parameters

A significant relationship was identified between BRAF mutation positivity and cutaneous MM and all non-cutaneous MM cases were identified to be BRAF negative ($p<0.0001$). There was no correlation between BRAF mutation status and other demographic, clinical and pathological features (Table 2).

Table 2. BRAF mutation status and correlation with other patient-tumor features

VARIABLES	**BRAF mutant**		**BRAF wild**		**p value**
	Number (n)	Percentage (%)	Number	Percentage	
Nodular MM	8	61.5	5	38.5	0.19
Other cutaneous MM	13	86.7	2	13.3	
LDH≥297.5	8	47.1	9	52.9	0.4
LDH<297.5	9	60	6	40	
Female	9	47.4	10	52.6	0.4
Male	12	60	8	40	
LN involvement	11	73.3	4	26.7	0.4
No LN involvement	5	55.6	4	44.4	
NLR≥1.97	9	45	11	55	0.2
NLR<1.97	10	66.7	5	33.3	
Metastatic stage	4	40	6	60	0.26
Early stage	17	65.4	9	34.6	
Skin emplacement	21	70	9	30	**<0.0001**
Non-skin emplacement	-	0	9	100	
Mitosis count≥5	5	45.5	6	54.5	0.4
Mitosis count<4	8	66.7	4	33.3	
Clark 4-5	9	60	6	40	0.39
Clark 1-2-3	9	81.8	2	18.2	

Ulceration present	8	53.3	7	46.7	0.21
Ulceration absent	9	81.8	2	18.2	
Recurrence present	13	59	9	41	0.6
Recurrence absent	6	50	6	50	
≥55 years	8	40	12	60	0.07
<55 years	13	68.4	6	31.6	

Correlation of NLR and Other Parameters

High NLR was identified to be significantly correlated with mitosis count (p=0.05), advanced stage (p=0.001), advanced age (p=0.008) and tumor ulceration (p=0.01). There were no correlations with serum LDH level, gender, Clark level, LN involvement, recurrence form and localization with NLR (Table 3.)

Table 3. Correlation of NLR values with demographic and clinicopathological features

Variables	**NLR<1.97**		**NLR≥1.97**		**p value**
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	
Cutaneous localization	35	52.2	32	47.3	0.3
Non-cutaneous localization	6	40	9	60	
Early stage	36	58.2	26	41.9	**0.001**
Metastatic stage	1	7.7	12	92.3	
<55	25	65.8	13	34.2	**0.008**
≥55	16	36.4	28	63.6	
Nodular type	9	47.4	10	52.6	0.3
Other cutaneous type	26	59.1	18	40.9	
Male	18	43.9	23	56.1	0.2
Female	23	56.1	18	43.9	
LN involvement absent	24	60	16	40	0.2
LN involvement present	9	45	11	55	
Clark ≥4	17	54.8	14	45.2	0.5
Clark ≤3	18	62.1	11	37.9	
Ulceration present	10	41.7	14	58.3	**0.01**
Ulceration absent	22	75.9	7	24.1	
LDH<297.5 U/L	18	51.4	17	48.6	0.6
LDH≥297.5 U/L	19	46.3	22	53.7	
Mitosis count ≤4	21	70	9	30	**0.05**
Mitosis count ≥5	9	42.9	12	57.1	
Regional recurrence	1	50	1	50	0.9
Recurrence as distant metastasis	9	52.9	8	47.1	
Recurrence as regional and distant metastasis	4	44.4	5	55.6	

3.2. Survival Analyses

Disease-free survival (DFS) analysis

DFS Analysis according to Stages and other Parameters

In early stage patients, median DFS duration was identified as 63 months. As stage increased, the DFS durations significantly shortened. There were no significant correlations between gender and age with DFS duration (p=0.19, p=0.8). DFS duration was shorter in the group with NLR ≥1.97, with no significant correlation identified

between NLR value and DFS duration (p=0.1). There was no significant difference in DFS according to LDH level at diagnosis (p=0.8).There was no significant correlation identified between BRAF mutation status and DFS duration (p=0.8).

Effect of Basic Pathologic Parameters on DFS

When the effect of basic pathologic parameters on DFS durations are analyzed, increased Breslow thickness (p=0.01), increased mitosis count (p=0.02), Clark level (p=0.05), and nodal involvement (p=0.03) were identified to be significantly correlated with shorter DFS durations. The presence of ulceration was associated with shorter DFS durations, but the difference was not statistically significant (p=0.09) (Table 3.4).

Overall Survival (OS) Analysis

OS Analysis according to Stages and other Parameters

The median overall survival duration was identified as 84 months (±27). The 1- and 5-year survival rates were identified as 90.7% and 59.9%, respectively. OS durations were 92 months for local stage, 45 months for stage III, and 13 months for stage IV (p<0.0001).There was no difference in OS durations in stage III patients according to adjuvant IFN use (p=0.6) .According to gender, OS durations were better in favor of women, though the OS difference was not statistically significant (p=0.12). According to age, patients younger than 55 years had a tendency toward longer overall survival compared to older patients; however, the difference was not statistically significant (p=0.06) .BRAF mutation was not observed to have an effect on OS durations (p=0.49). According to localization, there was no significant correlation identified between MM cases with cutaneous involvement and non-cutaneous malignant melanoma cases in terms of OS (p=0.36).

OS Analysis according to LDH Level

High LDH level at diagnosis was associated with short OS duration (p=0.04). Recurrence LDH levels were not identified to be significantly associated with OS duration (p=0.09) (Figure 2).

A

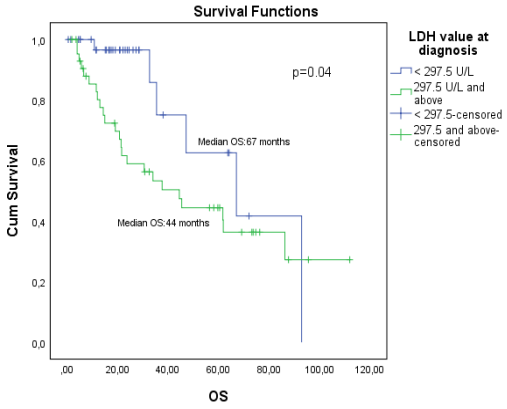

Figure 2 .A) OS curve according to LDH value at diagnosis,

OS Analysis according to NLR value

High NLR (≥1.97) was significantly correlated with short OS durations (p<0.0001) (Figure 3.). When OS durations are analyzed according to NLR separately for early stage and metastatic stage, high NLR in early stage patients was associated with short OS durations (mean 50 months vs 74 months) (p=0.02). In the group which had metastasis from the beginning, NLR was not observed to affect OS (p=0.39) (Figure 3)

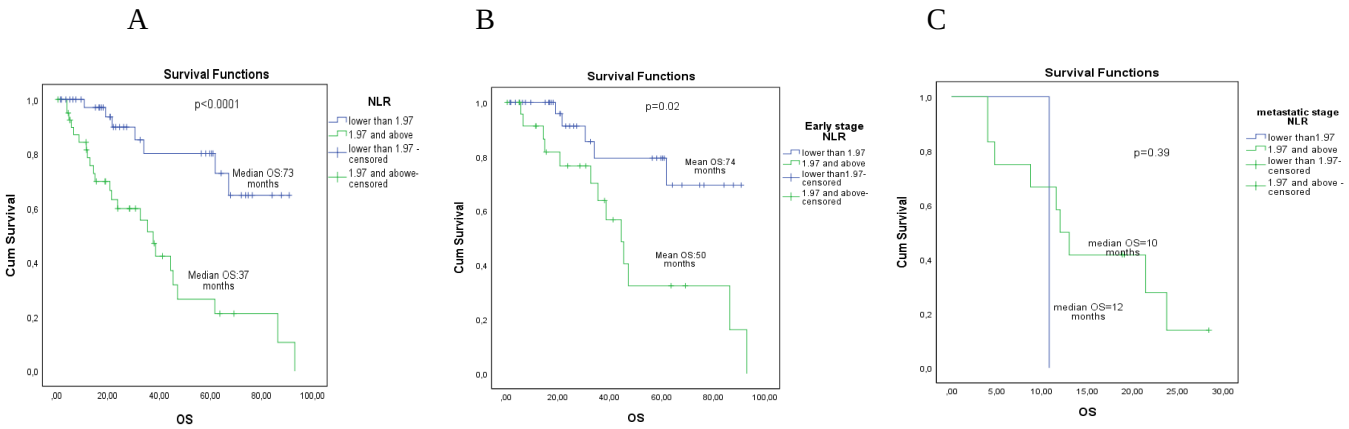

Figure 3. OS curves according to NLR value **A)** OS curves according to NLR value in all stages, **B)** OS curve according to NLR value in early stage, **C)** OS curve according to NLR value in metastatic stage

Effect of Basic Pathological Parameters on OS

When the effect of basic pathological parameters on OS is analyzed, increased Breslow thickness (p=0.01), increased mitosis count (p=0.02), Clark level (p=0.01), presence of ulceration (p=0.03), and nodal involvement (p=0.04) were identified to be correlated with significantly shorter OS durations (Figure 4) (Tablo 4.)

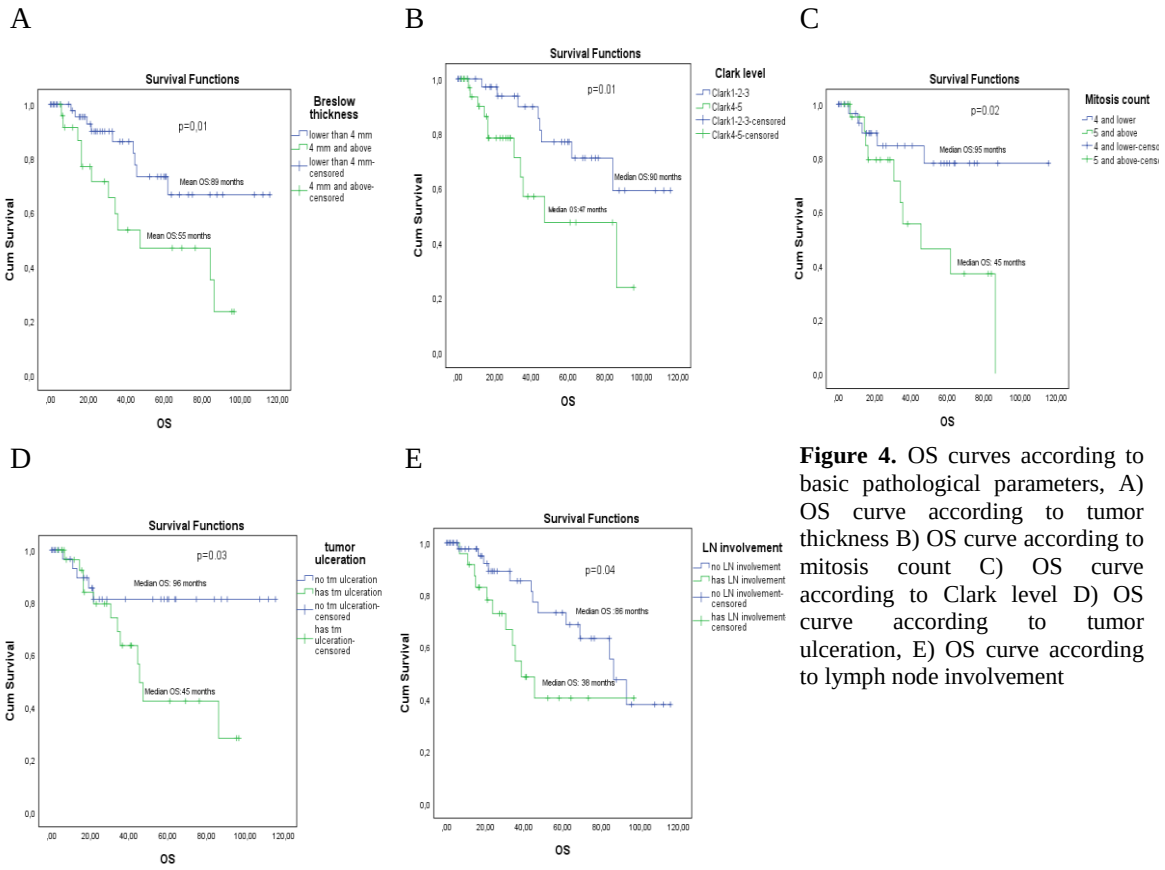

Figure 4. OS curves according to basic pathological parameters, A) OS curve according to tumor thickness B) OS curve according to mitosis count C) OS curve according to Clark level D) OS curve according to tumor ulceration, E) OS curve according to lymph node involvement

Table 4. Correlation of basic pathological parameters with OS and DFS

Correlation of pathological parameters and survival	OS	DFS
Breslow thickness	**0.01**	**0.01**
Mitosis	**0.02**	**0.02**
Clark level	**0.01**	**0.05**
Ulceration	**0.03**	0.09
LN involvement	**0.04**	**0.03**

OS: overall survival, DFS: disease-free survival

Multivariate Regression Analysis

Basic pathological parameters and other factors identified as significant for OS and DFS were included in Cox multivariate regression analysis. Accordingly, the most significant parameters for mortality were identified as nodal involvement and high NLR (HR; 4.6, p=0.01, HR: 3.4, p=0.04) (Table 5). The factor predicting recurrence best was identified as nodal involvement (HR: 3.2, p=0.03).

Table 5. Independent variables predicting mortality with Cox multivariate analysis

Independent variables	HR	P
Lymph node involvement	4.6(1.2-16.8)	**0.01**
NLR	3.4(1-11.9)	**0.04**
LDH level	2(0.5-7.9)	0.2
Breslow thickness	2.2(0.6-8.2)	0.2
Gender	0.4(0.08-2.9)	0.4
Mitosis	0.3(0.03-5.1)	0.4
Ulceration	0.4(0.03-4.9)	0.4

HR: Hazard ratio LDH: lactate dehydrogenase, NLR: neutrophil-lymphocyte ratio

Predictive Effect of NLR

There was no predictive effect of NLR in the metastatic patient group receiving 1st series medical treatment (Table 6).

Table 6. Treatment response rates according to NLR in metastatic patients

I. series treatment group	NLR<1.97	NLR≥1.97	P value
DSO	%40	%42.9	1
ORR	%40	%38.1	1

DSO: Disease Stabilization rate, ORR: Objective Response Rate

4. Discussion

There are few studies revealing the general clinicopathological profile of patients with melanoma diagnosis in Turkey and comparing with the world in general (10-14). As a result, this study aimed to reveal the general profile of MM patients attending Eskişehir

Osmangazi University Faculty of Medicine Medical Oncology Clinic and to compare the findings with data from Turkey and the world. Additionally, another important aim in the study was to investigate the correlation of NLR, predicted to be a new biomarker in recent years, with other clinicopathological parameters and its prognostic and predictive effect.

The mean age in our study was 55 years which is close to the median age data in the literature (15, 16). According to literature data, the most frequently observed MM type was superficial spreading melanoma (1, 2, 17). In our data, the most commonly observed type was nodular melanoma with surficial spreading melanoma observed with second-highest frequency. Several small population studies in Turkey reported the most commonly observed cutaneous melanoma subtype was nodular MM (10, 13). The reason for this difference compared to world data is considered to be genetic and ethnic differences.

According to the 2009-2015 SEER database, 84% of patients are local stage at time of diagnosis, 9% are stage III, 4% are metastatic stage and 4% have unknown stage (18). According to current SEER database data, the 5-year survival for all stages is 92%, while it is 99% for local disease, 65% for nodal disease and 25% in metastatic disease (19). In our study, the frequency and 5-year survival were similar to data from Turkey (10, 12) while the frequencies of nodal stage and metastatic stage at diagnosis were higher and survival rates were lower compared to the SEER database. The low survival rates according to stage in our study compared to literature data and the higher frequency of metastatic stage at diagnosis may be associated with the limited number of patients and the attendance of patients in later periods.

According to literature data, the most frequent metastasis regions were lung, lymph node metastasis, skin metastasis and brain metastasis (12, 20-22). In our study, the most frequent metastasis localizations were lung metastasis, distant lymph node metastasis and liver metastasis, in order.

LDH isoenzymes play roles in both glycolysis and oxidative phosphorylation. Melanoma cells have more active oxidative phosphorylation, glycolysis and lactate metabolism and proteins like LDH isoenzymes playing a role in stages of these cycles are shown to be increased in melanoma cells. High serum LDH level is a reflection of high LDH isoenzymes in peripheral blood (23). LDH level is a marker included in MM staging (24, 25) with proven prognostic effect (26-30). In our study, a correlation was identified between high LDH levels and poor overall survival, but there was no significant correlation in terms of disease-free survival.

After the role of BRAF mutations in melanoma pathogenesis was understood and included in treatment, a predictive effect was naturally revealed. Many studies have researched whether it has prognostic effect or not. According to literature data, BRAF mutation positivity is frequently encountered in cutaneous melanoma, while BRAF mutation positivity is rare in non-cutaneous melanoma (31-34). A range of studies in the literature have identified BRAF mutation status is associated with increased Breslow thickness, high mitosis count, presence of ulceration, advance stage, smoking habit, male sex, young age, tumor type, superficial spreading melanoma type, nodular melanoma type and localization on the body (35-38). A study by Frauchiger et al. did not identify a difference between survival of patients with BRAF mutant and BRAF wild type (39). A meta-analysis study by Lars Ny et al. reported the presence of BRAF mutation was associated with poor survival (40). In our study, no significant effect on survival was identified for BRAF mutation. Additionally, when correlations between BRAF mutation status with demographic and clinicopathological features are evaluated, all non-cutaneous malignant melanoma cases with BRAF examined were identified to be BRAF negative. There were no significant relationships between BRAF mutation status with other parameters.

It is known that the inflammatory response forming around a tumor is reflected in a range of laboratory parameters like basal leukocyte values and subtypes in blood, CRP, fibrinogen

and NLR. As a result of interactions between cytokines produced by melanoma cells with receptors expressed in neutrophils, neutrophils are stimulated and are thought to play an effective role in tumoral angiogenesis, tumoral invasion and metastasis mediated by anti-VEGF (3, 41). Lymphocytes induce cytotoxic cell death and suppress tumor cell proliferation and progression showing anti-tumoral effect and immunity (42). Additionally, lymphocytes were shown to have preventive effect on migration of tumor cells (43). As a result, the elevation in neutrophils and a fall in lymphocyte levels in peripheral blood may be associated with poor prognosis. Studies have shown that high NLR values are associated with poor survival in melanoma and NLR is thought to have prognostic effect for survival (44-47). Studies in recent years about NLR have generally taken the NLR cut-off value as between 2-5 determined with the ROC method and a variety of other methods (47-49). Our NLR value was determined as the median value of 1.97. When the ROC curve is examined, this value was identified as a significant cut-off value. In the literature, several meta-analysis studies in recent years have identified high NLR is associated with poor survival (44-50). In our study, the NLR in the metastatic stage at diagnosis did not have prognostic effect, while high NLR in the early stage and general population was identified to be associated with short OS durations. In our study, it may be considered that the prognostic effect of NLR on OS in the early stage was reflected in the general population as the population was dominated by patients presenting in the early stage at time of diagnosis.

There are a range of studies researching the predictive effect of NLR in the literature in recent times. A study by Khoja et al. did not identify a significant difference in terms of treatment response according to basal NLR, while a difference was identified at the end of treatment in terms of basal NLR (51). Two studies in recent times about PD-1 inhibitors identified high NLR was associated with poor treatment response (52, 53). As our cases had attended the clinic from 2010-2017 and BRAF-MEK inhibitors were only licensed in Turkey in 2015, cases in the study group received systemic chemotherapy and BRAF-MEK inhibitors in the first stage. NLR was not identified to have a predictive effect creating a significant difference for the metastatic patient group receiving first stage treatment.

Several studies researched the correlation between NLR elevation and other demographic and clinicopathological features and identified NLR was associated with advanced age, male sex, increased Breslow thickness and high mitotic ratio (54, 55). In our study, high NLR was identified to be correlated with advanced age, advanced stage, presence of ulceration and high mitosis counts. Additionally, when the correlations between stage and other demographic and clinicopathological features are investigated, high NLR was identified to be correlated with more advanced stage for tumors with advanced stage and non-cutaneous MM type, consistent with literature data (56).

As is known, Breslow thickness, presence of ulceration, mitosis, Clark level, and nodal involvement are prognostic factors with significant effects on survival in the AJCC staging system taking TNM staging and in large population studies. A range of studies in recent times have identified high NLR, advanced age, male sex, presence of ulceration, lymphovascular invasion, sentinel lymph node involvement, anatomic localization, high LDH levels and metastasis localization are the most effective parameters on mortality in multivariate analyses. Additionally, the parameters predicting recurrence most were reported to be factors like Breslow thickness, presence of ulceration, SLN involvement and age (50, 53, 54, 57-59). In our study, correlations were identified between Breslow thickness, presence of ulceration, Clark level, mitosis count and LN involvement with poor overall survival. Increased Breslow thickness, Clark level, mitosis count and LN involvement were also associated with poor disease-free survival. Cox multivariate regression analysis identified that the parameters with most significant effect on mortality were nodal involvement and high NLR. Additionally, the independent

parameter that predicted recurrence most was identified as nodal disease.

In this study some basic results were obtained: The most commonly observed melanoma type was cutaneous melanoma (80.4%, n=86), with the most commonly identified subtype nodular MM.BRAF mutation status was not observed to affect survival. In addition to classic pathological parameters for survival, high NLR and high LDH level were identified to be associated with short OS duration. The NLR cut-off value was calculated as the median value and identified as 1.97. Additionally, in the early stage patient group a correlation of NLR with short OS durations was identified, with NLR not identified to effect OS in the metastatic stage. Cox multivariate regression analysis identified the most significant parameters increasing mortality risk were high NLR and nodal involvement. Additionally, the parameter predicting recurrence best was identified as nodal involvement. NLR was not identified to have predictive effect on the patient group receiving 1st series treatment in the metastatic stage.

In spite of all these significant results, our study has some limitations. These limitations include the study being based on single-center experience, the low number of patients, and late licensing of standard treatments in our country for melanoma patients and lack of effective use. In conclusion, it appears that the simple laboratory parameter of NLR may be a prognostic marker along with classic pathological parameters. There is a need for multicenter studies assessing factors like geography and ethnic group and their effect on NLR. We think that our study will contribute to Turkish and world data by including these features.

REFERENCES

1. Matthews NH, Li W-Q, Qureshi AA, Weinstock MA, Cho E. Epidemiology of melanoma. Cutaneous Melanoma: Etiology and Therapy. Codon Publications; 2017;3-22.
2. Linos E, Swetter SM, Cockburn MG, Colditz GA, Clarke CAJJoID. Increasing burden of melanoma in the United States. 2009;129(7):1666-74.
3. Dhawan P, Richmond A. Role of CXCL1 in tumorigenesis of melanoma. Journal of leukocyte biology. 2002;72(1):9-18.
4. Genomic Classification of Cutaneous Melanoma. Cell. 2015;161(7):1681-96.
5. Ascierto PA, McArthur GA, Dréno B, Atkinson V, Liszkay G, Di Giacomo AM, et al. Cobimetinib combined with vemurafenib in advanced BRAFV600-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. 2016;17(9):1248-60.
6. Long G, Flaherty K, Stroyakovskiy D, Gogas H, Levchenko E, De Braud F, et al. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. 2017;28(7):1631-9.
7. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Science translational medicine. 2012;4(127):127ra37.
8. Robert C, Ribas A, Hamid O, Daud A, Wolchok JD, Joshua AM, et al. Three-year overall survival for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. American Society of Clinical Oncology; 2016.
9. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob J-J, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. 2017;377(14):1345-56.
10. Uysal-Sonmez O, Tanriverdi O, Esbah O, Uyeturk U, Helvaci K, Bal O, et al. Multicenter evaluation of patients with cutaneous malignant melanoma in Turkey: MELAS study. 2013;14(1):533-7.
11. Abali H, Celik I, Karaca B, Turna H, Kaytan ES, Akman T, et al. Cutaneous melanoma in Turkey: analysis of 1157 patients in the Melanoma Turkish Study. 2015;20(4):1137-41.
12. Tas F, Kurul S, Camlica H, Topuz EJJjoco. Malignant melanoma in Turkey: a single institution's experience on 475 cases. 2006;36(12):794-9.
13. Sula B, Uçmak F, Kaplan MA, Urakçi Z, Arica M, Isikdogan AJPAMJ. Epidemiological and clinical characteristics of malignant melanoma in Southeast Anatolia in Turkey. 2016;24(1).
14. Baykal C, Atci T, Akay BNJJoOS. Is the frequency of primary cutaneous melanoma increasing in Turkey? An evaluation of the experiences of two dermatology centers. 2018;4(1):19-23.

15. Rastrelli M, Tropea S, Rossi CR, Alaibac MJIv. Melanoma: epidemiology, risk factors, pathogenesis, diagnosis and classification. 2014;28(6):1005-11.
16. Batzler WU, Giersiepen K, Hentschel S, Husmann G, Kaatsch P, Katalinic A, et al. Cancer in Germany 2003-2004 Incidence and Trends. 2008.
17. Chang AE, Karnell LH, Menck HRJCIIJotACS. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. 1998;83(8):1664-78.
18. NIH(National Cance İnstitute), Surveillance ,Epidemiology, and Results Programme(SEER) [online]. Available from: /seer.cancer.gov/.
19. Siegel RL, Miller KD, Jemal AJCACJfC. Cancer statistics, 2020. 2020;70(1):7-30.
20. Meyer T, Merkel S, Goehl J, Hohenberger WJCIIJotACS. Surgical therapy for distant metastases of malignant melanoma. 2000;89(9):1983-91.
21. Leung AM, Hari DM, Morton DLJCj. Surgery for distant melanoma metastasis. 2012;18(2):176.
22. Francken AB, Accortt NA, Shaw HM, Wiener M, Soong S-j, Hoekstra HJ, et al. Prognosis and determinants of outcome following locoregional or distant recurrence in patients with cutaneous melanoma. 2008;15(5):1476-84.
23. Ho J, de Moura MB, Lin Y, Vincent G, Thorne S, Duncan LM, et al. Importance of glycolysis and oxidative phosphorylation in advanced melanoma. 2012;11(1):76.
24. Gershenwald JE, Scolyer RA, Hess KR, Sondak VK, Long GV, Ross MI, et al. Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. 2017;67(6):472-92.
25. Balch CM, Gershenwald JE, Soong S-j, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. 2009;27(36):6199.
26. Sirott MN, Bajorin DF, Wong GY, Tao Y, Chapman PB, Templeton MA, et al. Prognostic factors in patients with metastatic malignant melanoma: a multivariate analysis. 1993;72(10):3091-8.
27. Gao D, Ma X. Serum lactate dehydrogenase is a predictor of poor survival in malignant melanoma. Panminerva medica. 2017;59(4):332-7.
28. Schmidt H, Bastholt L, Geertsen P, Christensen IJ, Larsen S, Gehl J, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. 2005;93(3):273.
29. Schmidt H, Suciu S, Punt CJ, Gore M, Kruit W, Patel P, et al. Pretreatment levels of peripheral neutrophils and leukocytes as independent predictors of overall survival in patients with American Joint Committee on Cancer Stage IV Melanoma: results of the EORTC 18951 Biochemotherapy Trial. 2007;25(12):1562.
30. Manola J, Atkins M, Ibrahim J, Kirkwood J. Prognostic factors in metastatic melanoma: a pooled analysis of Eastern Cooperative Oncology Group trials. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2000;18(22):3782-93.
31. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417(6892):949-54.
32. Rabbie R, Ferguson P, Molina-Aguilar C, Adams DJ, Robles-Espinoza CDJTJop. Melanoma subtypes: genomic profiles, prognostic molecular markers and therapeutic possibilities. 2019;247(5):539-51.
33. Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature. 2009;457(7229):599-602.
34. Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, et al. Mutations in GNA11 in uveal melanoma. The New England journal of medicine. 2010;363(23):2191-9.
35. Sehdev A, Hayden R, Kuhar MJ, Cheng L, Warren SJ, Mark LA, et al. Prognostic role of BRAF mutation in malignant cutaneous melanoma. American Society of Clinical Oncology; 2018.
36. Kim SY, Kim SN, Hahn HJ, Lee YW, Choe YB, Ahn KJJJotAAoD. Metaanalysis of BRAF mutations and clinicopathologic characteristics in primary melanoma. 2015;72(6):1036-46. e2.
37. Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, et al. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011;29(10):1239-46.
38. Lee HW, Song KH, Hong JW, Jeon SY, Ko DY, Kim KH, et al. Frequency of BRAF mutation and clinical relevance for primary melanomas. 2012;46(3):246.
39. Frauchiger AL, Mangana J, Rechsteiner M, Moch H, Seifert B, Braun R, et al. Prognostic relevance of lactate dehydrogenase and serum S100 levels in stage IV melanoma with known BRAF mutation status. 2016;174(4):823-30.
40. Ny L, Nyakas M, Hernberg M, Koivunen J, Oddershede L, Yoon M-R, et al. BRAF mutation as a prognostic marker for survival in malignant melanoma: A systematic review and meta-analysis. American Society of Clinical Oncology; 2018;371(20):1877-1888

41. Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss SJTJoci. Neutrophils responsive to endogenous IFN-β regulate tumor angiogenesis and growth in a mouse tumor model. 2010;120(4):1151-64.
42. Yang Z, Gu J-H, Guo C-S, Li X-H, Yang W-CJO. Preoperative neutrophil-to-lymphocyte ratio is a predictor of survival of epithelial ovarian cancer: a systematic review and meta-analysis of observational studies. 2017;8(28):46414.
43. Marchioni M, Cindolo L, Autorino R, Primiceri G, Arcaniolo D, De Sio M, et al. High neutrophil-to-lymphocyte ratio as prognostic factor in patients affected by upper tract urothelial cancer: a systematic review and meta-analysis. 2017;15(3):343-9. e1.
44. Cassidy MR, Wolchok RE, Zheng J, Panageas KS, Wolchok JD, Coit D, et al. Neutrophil to lymphocyte ratio is associated with outcome during ipilimumab treatment. 2017;18:56-61.
45. Finon A, Zaragoza J, Maillard H, Beneton N, Bens G, Samimi M, et al. A high neutrophil to lymphocyte ratio prior to BRAF inhibitor treatment is a predictor of poor progression-free survival in patients with metastatic melanoma. 2018;28:38-43.
46. Rosner S, Kwong E, Shoushtari AN, Friedman CF, Betof AS, Brady MS, et al. Peripheral blood clinical laboratory variables associated with outcomes following combination nivolumab and ipilimumab immunotherapy in melanoma. 2018;7(3):690-7.
47. Ding Y, Zhang S, Qiao JJM. Prognostic value of neutrophil-to-lymphocyte ratio in melanoma: Evidence from a PRISMA-compliant meta-analysis. 2018;97(30).
48. Zhan H, Ma J-Y, Jian Q-CJCCA. Prognostic significance of pretreatment neutrophil-to-lymphocyte ratio in melanoma patients: A meta-analysis. 2018.
49. Ma J, Kuzman J, Ray A, Lawson BO, Khong B, Xuan S, et al. Neutrophil-to-lymphocyte Ratio (NLR) as a predictor for recurrence in patients with stage III melanoma. 2018;8(1):4044.
50. Lino-Silva LS, Salcedo-Hernández RA, García-Pérez L, Meneses-García A, Zepeda-Najar CJMr. Basal neutrophil-to-lymphocyte ratio is associated with overall survival in melanoma. 2017;27(2):140-4.
51. Khoja L, Atenafu EG, Templeton A, Qye Y, Chappell MA, Saibil S, et al. The full blood count as a biomarker of outcome and toxicity in ipilimumab-treated cutaneous metastatic melanoma. 2016;5(10):2792-9.
52. Nakamura Y, Tanaka R, Maruyama H, Ishitsuka Y, Okiyama N, Watanabe R, et al. Correlation between blood cell count and outcome of melanoma patients treated with anti-PD-1 antibodies. 2019;49(5):431-7.
53. Hemadri A, Lin H, Lin Y, Rose A, Sander C, Najjar Y, et al. Association of baseline neutrophil-to-lymphocyte ratio (NLR) with response and survival in advanced melanoma (MEL) receiving PD-1 inhibitors. American Society of Clinical Oncology; 2019.
54. Davis JL, Langan RC, Panageas KS, Zheng J, Postow MA, Brady MS, et al. Elevated blood neutrophil-to-lymphocyte ratio: a readily available biomarker associated with death due to disease in high risk nonmetastatic melanoma. 2017;24(7):1989-96.
55. Bayoglu IV, Kurtel G, Alacacioglu A, Varim C, Yildiz I, Kucukzeybek Y, et al. Can basal neutrophil/lymphocytes ratio predict clinical outcome of melanoma patients? : American Society of Clinical Oncology; 2015.
56. Grözinger G, Mann S, Mehra T, Klumpp B, Grosse U, Nikolaou K, et al. Metastatic patterns and metastatic sites in mucosal melanoma: a retrospective study. 2016;26(6):1826-34.
57. Balch CM, Soong S-j, Ross MI, Urist MM, Karakousis CP, Temple WJ, et al. Long-term results of a multi-institutional randomized trial comparing prognostic factors and surgical results for intermediate thickness melanomas (1.0 to 4.0 mm). 2000;7(2):87-97.
58. Capone M, Giannarelli D, Mallardo D, Madonna G, Festino L, Grimaldi AM, et al. Baseline neutrophil-to-lymphocyte ratio (NLR) and derived NLR could predict overall survival in patients with advanced melanoma treated with nivolumab. Journal for immunotherapy of cancer. 2018;6(1):74.
59. Mays MP, Martin RC, Burton A, Ginter B, Edwards MJ, Reintgen DS, et al. Should all patients with melanoma between 1 and 2 mm Breslow thickness undergo sentinel lymph node biopsy? 2010;116(6):1535-44.

Etik Bilgiler
Etik Kurul Onayı: Çalışma Eskişehir Osmangazi Üniversitesi Girişimsel Olmayan Etik Kurulu'ndan tarafından onaylanmıştır (Karar no: 14, Tarih: 21.11.2017).
Onam: Bu çalışma için katılımcılardan sözlü onam alınmıştır.
Telif Hakkı Devir Formu: Tüm yazarlar tarafından Telif Hakkı Devir Formu imzalanmıştır.
Hakem Değerlendirmesi: Hakem değerlendirmesinden geçmiştir.
Yazar Katkı Oranları: Fikir/kavram: L.D YSK Tasarım: YSK Veri Toplama: YSK, LD. BY, MD. Veri İşleme: YSK, LD, MD, BY. Analiz/Yorum: LD, YSK, BY, MD. Literatür taraması: YSK, LD, Yazma: YSK, LD.
Çıkar Çatışması Bildirimi: Yazarlar çıkar çatışması olmadığını beyan etmişlerdir.
Destek ve Teşekkür Beyanı: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.