

## ORIGINAL ARTICLE

# Are neutrophil / lymphocyte ratio and platelet / lymphocyte ratio associated with prognosis in patients with HER2-positive early breast cancer receiving adjuvant trastuzumab?

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## Summary

**Purpose:** To investigate whether the pretreatment neutrophil lymphocyte ratio (NLR) and the platelet lymphocyte ratio (PLR) have any prognostic significance in patients with HER2-positive early breast cancer receiving adjuvant trastuzumab.

**Methods:** 187 patients were retrospectively analyzed. The patients were separated into two groups according to the mean value of NLR and PLR (low NLR  $\leq 2.38$ , high NLR  $> 2.38$ ; and low PLR  $\leq 161.28$ , high PLR  $> 161.28$ , respectively). The relationship between pretreatment NLR, PLR and clinicopathological factors was investigated. Univariate and multivariate Cox regression analyses were performed. To evaluate survival rates, the Kaplan–Meier method with log rank test were used.

**Results:** The median duration of follow up was 26.0 months (range 6.0-84.0). In high NLR and PLR groups, the mean age was lower, tumor size was larger and the number of hormone receptor positive patients was higher. No statistically significant relationship was found between clinicopathological factors and both NLR and PLR groups. During follow up, the

rate of relapse was 12.6% in the low NLR group, 16.2 % in the high NLR group, 12.6% in the low PLR group and 15.8% in the high PLR group ( $p$ =non significant). Although median disease free survival (DFS) was shorter in the high NLR than in the low NLR group, the difference was not statistically significant ( $p=0.45$ ). No statistically significant difference was found between high and low PLR groups with regard to median DFS and overall survival (OS) ( $p=0.76$ ,  $p=0.29$ , respectively).

**Conclusion:** We conclude that in HER2-positive early breast cancer patients receiving adjuvant trastuzumab with high pretreatment NLR, DFS was shorter. As for PLR, no effect either on DFS or on OS was registered. Prospective studies with larger number of patients are required in order to evaluate the prognostic effect of NLR and PLR in HER2-positive breast cancer patients.

**Key words:** breast cancer, HER2 positive, lymphocytes, neutrophil, platelet

## Introduction

Breast cancer is a heterogeneous disease with diverse natural course and response to treatment. The human epidermal growth factor receptor2 (HER2) gene is amplified, overexpressed, or both in 15-25% of breast cancers and is associated with aggressive disease [1-3]. Trastuzumab is a human-

ized monoclonal antibody developed against the extracellular domain of HER2 and has established clinical benefits in women with HER2-positive breast cancer in metastatic and early disease settings [4-8].

Inflammation is an important component of

tumor progression. Most cancers develop at infection regions such as chronic irritation and inflammation. Tumor microenvironment regulated by inflammatory cells clearly plays a basic role in the neoplastic process, stimulation of proliferation and in migration and survival [9]. Recent studies have demonstrated that peripheral blood absolute neutrophil count/absolute lymphocyte count ratio (ANC/ALC, or the NLR) can be used as a prognostic indicator in malignant tumors. High NLR has been reported to be a poor prognostic factor for survival in gastric cancer, renal cell cancer, metastatic melanoma, and advanced non-small-cell lung cancer (NSCLC) [10-14]. Platelets may release some growth factors such as platelet derived growth factor (PDGF), platelet factor 4 (PF4) and thrombospondin [15,16]. It has been demonstrated that these factors induce hematogenous tumor spread, lead to tumor cell adhesion and invasion and play an important part in tumor progression [17]. Pretreatment PLR was found to be associated with poor prognosis in patients with epithelial ovarian cancer, oesophageal cancer and lung cancer [18-20].

Previous studies demonstrated that infiltration of tumor by lymphocytes improves prognosis in breast cancer patients [21]. Especially, CD8+ lymphocyte infiltrated tumor was shown to be associated with improvement in survival in breast cancer patients [22]. In recent studies on breast cancer, it has been shown that survival was shorter in patients with high NLR values [23-25] and it has been proposed that increase in neutrophils elicits tumor growth and metastasis [26,27]. Inhibition of lymphocyte activity was found to be associated with antitumor response [28,29].

To the best of our knowledge, the present study is the first to investigate early stage HER2-positive breast cancer patients receiving adjuvant trastuzumab, in whom NLR and PLR have been evaluated as prognostic factors. In adjuvant trastuzumab studies, a tumor size over 2cm, lymph node positivity, and ER positivity were found to be factors predictive of DFS [30-32]. There is no previous study considering the role of host immunity (lymphocytes) and inflammatory response (i.e. neutrophils, platelets) in early stage HER2-positive breast cancer patients receiving adjuvant trastuzumab.

The aim of the present study was to investigate whether there was any prognostic significance of pretreatment NLR and PLR in patients with HER2-positive early breast cancer receiving adjuvant trastuzumab.

## Methods

This study was carried out in the oncology departments of two centers (Bursa Oncology hospital and Balıkesir Public hospital, in Turkey) after approval of the ethics committee. HER2-positive early breast cancer patients diagnosed between January 2009 and January 2014, who underwent resection and received adjuvant trastuzumab, were retrospectively evaluated. HER2 was determined by immunohistochemistry (IHC) and scored from 0 to 3+. Scoring 2+ was assigned when there was weak to moderate complete membrane staining in >10% of tumor cells, whereas scoring 3+ consisted of uniform intense membrane staining of >10% of tumor cells. Silver in situ hybridization (SISH) or fluorescence in situ hybridization (FISH) analyses were carried out for all HER2 IHC 2+ samples. Of 210 patients 187 (89%) whose blood neutrophil and lymphocyte numbers were recorded before treatment were included in the study.

Patients who had metastasis at the time of diagnosis, those with contralateral breast cancer or cancer other than breast cancer, those who received neoadjuvant treatment, who had evidence of clinically active infection, those with hematological, chronic inflammatory or autoimmune diseases, and patients who were on active steroid treatment were excluded from the study. Patient demographic characteristics, histopathological data, ER and PR status, laboratory parameters, surgery, site of first relapse and time of relapse, adjuvant chemotherapy and radiotherapy were recorded. Staging of the patients was carried out according to the American Joint Committee on Cancer (AJCC) criteria.

Prior to treatment, complete blood count including total white blood cells, neutrophils, lymphocytes, and platelets was recorded. NLR and PLR were calculated as the ratio of the neutrophils and platelets to lymphocytes. Mean value was used for NLR and PLR because distribution was normal. The patients were divided into two groups according to the mean value of NLR and PLR (low NLR:  $\leq 2.38$ , high: NLR:  $> 2.38$ ; and low PLR:  $\leq 161.28$ , high PLR:  $> 161.28$ , respectively).

In all patients, adjuvant trastuzumab-based treatment was planned for one year. Adjuvant trastuzumab was administered every three weeks in patients with primary breast cancer following surgery, adjuvant chemotherapy and radiotherapy (if applicable). Trastuzumab treatment was commenced with 8 mg/kg loading dose followed by 6 mg/kg maintenance dose every three weeks for one-year.

Relapses were registered as local, contralateral, or distant. DFS was defined as the time from diagnosis to local, regional, or distant relapse or death from breast cancer. OS was defined as the time from diagnosis to death or last follow-up contact.

## Statistics

Statistical data were analysed using SPSS 15.0

software program (SPSS Inc., Chicago, Ill, USA). Fisher's and chi square tests were used for nominal variables and numerical data and Kaplan-Meier analysis was employed for survival rates with comparisons made with log-rank test. Univariate and multivariate analyses were performed to statistically define prognostic factors. A p value <0.05 was considered statistically significant.

## Results

### Patient characteristics

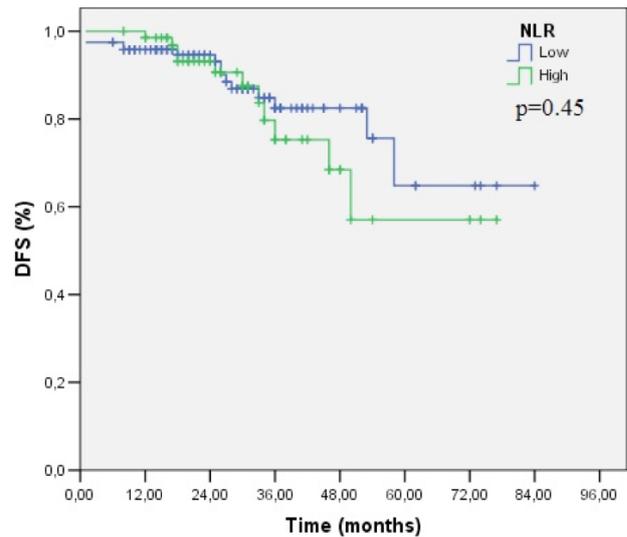
The number of patients with IHC score 3+ was 138 (73.8%). The number of patients with IHC score 2+ who were found to be HER2 amplification positive with FISH or SISH analysis was 49 (26.2%). The mean patient age was  $51.4 \pm 10.7$  years. Radical mastectomy was carried out on 72.7% of the patients, 38.5% of patients had negative nodes, 31% had T1 tumor size and 51.3% had positive ER. The rate of either ER or PR positivity was 53.5%. Table 1 lists the characteristics of patients and tumors.

Of 187 patients, 170 (90%) completed the planned adjuvant one-year trastuzumab treatment. The treatment was interrupted in 17 patients due to relapse during treatment. Of the patients, 74.3% received taxane- and anthracycline-based chemotherapy, 23.5% received only anthracycline-based chemotherapy and 2.1% only adjuvant taxane and trastuzumab treatment. Radiotherapy following mastectomy was delivered to 79.1% of the patients, while 35.3% of the patients received radiotherapy along with trastuzumab. Finally, 53.5% of the patients received hormone treatment.

During a median follow-up of 26.0 months (range 6.0-84.0), relapse developed in 26 (13.9%) patients. In these patients, the initial sites of metastasis were as follows: brain (3.8%), bone (3.8%), liver (2.6%), lung (2.6%), and skin (1.2%). In addition, there was also thyroid metastasis concurrent with brain metastasis in one patient.

### Pattern of NLR and PLR distribution

The mean NLR value was  $2.38 \pm 1.42$  and mean PLR value  $161.28 \pm 73.68$ . The mean age was lower in the groups with high PLR (high PLR:  $>161.28$ ) than that in the groups with low PLR (low PLR:  $\leq 161.28$ ) ( $p=0.02$ ). Although the mean age in the high NLR group (NLR:  $>2.38$ ) was lower than that in the low NLR group (NLR:  $\leq 2.38$ ), the difference was not statistically significant. The majority of



**Figure 1.** Disease free survival by neutrophil /lymphocyte ratio during diagnosis.

patients with tumor size  $>20$ - $\leq 50$  mm belonged in the high NLR group while the majority of patients with tumor size  $>50$  mm belonged in the high PLR groups, but the difference was not statistically significant. In hormone receptor-positive patients, the number of patients with high NLR and high PLR was high, but the difference with hormone receptor negative patients was not statistically significant. Also, no significant correlations between NLR and clinicopathological factors were seen. Characteristics of the patients according to NLR and PLR groups are shown in Table 1.

### Univariate and multivariate analysis for clinicopathological factors

In univariate analysis performed for DFS, no statistically significant relation with age, menopausal status, surgical method, histopathology, nodal involvement, estrogen receptor status, progesterone receptor status and hormone receptor status was found but larger tumor size and stage III were significantly associated ( $p<0.05$ ) with DFS. In univariate analysis for OS, lymph node positivity, grade III differentiation and stage III were significantly associated with OS ( $p<0.05$ ) (Table 2). Multivariate analysis didn't reveal any independent prognostic factor for both DFS and OS.

**Table 1.** Patients characteristics according to NLR and PLR groups

Variables	N(%)	NLR		p value	PLR		p value
		Low (N:119) N (%)	High (N:68) N (%)		Low (N:111) N (%)	High (N:76) N (%)	
Age (years)							
Mean age ±SD	51.4±10.4	51.9 ± 10.4	50.4 ± 10.4	0.35	52.8 ± 10.0	49.3 ± 10.6	0.02
Menopause							
Pre	54 (28.9)	34 (36.8)	20 (29.4)	0.99	30 (27.0)	24 (31.6)	0.51
Post	133 (71.1)	85 (71.4)	48 (70.6)		81 (73.0)	52 (68.4)	
Surgery							
Lumpectomy	51 (27.3)	35 (29.4)	16 (23.5)	0.49	34 (30.6)	17 (22.4)	0.24
Mastectomy	136 (72.7)	84 (70.6)	52 (76.5)		77 (69.4)	59 (77.6)	
Stage							
IA	14 (7.5)	10 (8.4)	4 (5.9)	0.81	10 (9.0)	4 (5.3)	0.85
IB	6 (3.2)	5 (4.2)	1 (1.5)		4 (3.6)	2 (2.6)	
IIA	66 (35.3)	40 (33.6)	26 (38.2)		41 (36.9)	25 (32.9)	
IIB	46 (24.6)	27 (22.7)	19 (27.9)		25 (22.5)	21 (27.6)	
IIIA	27 (14.4)	19 (19.0)	8 (11.8)		14 (12.6)	13 (17.1)	
IIIB	12 (6.4)	7 (5.9)	5 (7.4)		8 (7.2)	4 (5.3)	
IIIC	16 (8.6)	11 (9.2)	5 (7.4)		9 (8.1)	7 (9.2)	
Histopathology							
Invasive ductal carcinoma	170 (90.9)	107 (89.9)	63 (92.6)	0.61	102 (91.9)	68 (89.5)	0.61
Other*	17 (9.1)	12 (10.1)	5 (7.4)		9 (8.1)	8 (10.5)	
Tumor size							
T1	58 (31.0)	41 (35.5)	17 (25.0)	0.17	36 (32.4)	22 (28.9)	0.26
T2	116 (62.0)	68 (57.1)	48 (70.6)		70 (63.1)	46 (60.5)	
≥T3	13 (7.0)	10 (8.4)	3 (4.4)		5 (4.5)	8 (10.5)	
Number of lymph node metastasis							
0	72 (38.5)	43 (37.1)	29 (42.6)	0.60	43 (39.8)	29 (38.2)	0.68
1-3	54 (28.9)	34 (29.3)	20 (29.4)		34 (31.5)	20 (26.3)	
4-9	34 (18.2)	21 (18.1)	13 (19.1)		17 (15.7)	17 (22.4)	
>9	24 (12.8)	18 (15.5)	6 (8.8)		14 (13.0)	10 (13.2)	
Unknown	3	3	0		3	0	
Estrogen receptor							
Negative	91 (48.7)	58 (48.7)	33 (48.5)	0.99	56 (50.5)	35 (46.1)	0.65
Positive	96 (51.3)	61 (51.3)	35 (51.5)		55 (49.5)	41 (53.9)	
Progesterone receptor							
Negative	112 (59.9)	74 (62.2)	38 (55.9)	0.44	69 (62.2)	43 (56.6)	0.45
Positive	75 (40.1)	45 (37.8)	30 (44.1)		42 (37.8)	33 (43.4)	
Hormone receptor							
ER and PR negative	100 (53.5)	55 (46.2)	32 (47.1)	0.92	54 (48.6)	33 (43.4)	0.55
ER and /or PR positive	87 (46.5)	64 (53.8)	36 (52.9)		57 (51.4)	43 (56.6)	
Histologic grade							
1	5 (2.7)	1 (0.9)	4 (6.3)	0.41	2 (1.9)	3 (4.5)	0.77
2	69 (36.9)	45 (41.7)	24 (38.1)		43 (41.3)	26 (38.8)	
3	97 (51.9)	62 (57.4)	35 (55.6)		59 (56.7)	38 (56.7)	
Unknown	6	1	5		7	9	
Chemotherapy							
Anthracyclines	44 (23.5)	27 (22.7)	17 (25.0)	0.84	28 (25.2)	16 (21.1)	0.76
Taxanes	4 (2.1)	3 (2.5)	1 (1.5)		2 (1.8)	2 (2.6)	
Anthracyclines + Taxanes	139 (74.3)	89 (74.8)	50 (73.5)		81 (73.0)	58 (76.3)	
Hormonotherapy							
No	87 (46.5)	56 (47.1)	31 (45.6)	0.98	53 (47.7)	34 (44.7)	0.92
Tamoxifen	43 (23)	27 (22.7)	16 (23.5)		25 (22.5)	18 (23.7)	
Aromatase inhibitors	57 (30.5)	36 (30.3)	21 (30.9)		33 (29.7)	24 (31.6)	
Radiotherapy							
No	39 (20.9)	21 (17.6)	18 (26.5)	0.19	23 (20.7)	16 (21.1)	0.99
Yes	148 (79.1)	98 (82.4)	50 (73.5)		88 (79.3)	60 (78.9)	
Relapse							
Yes	26 (13.9)	15 (12.6)	11 (16.2)	0.52	14 (12.6)	12 (15.8)	0.67
No	161 (86.1)	104 (87.4)	57 (83.8)		97 (87.4)	64 (84.2)	

\*mixed tumors, medullary tumors; histologic grade: 1= well differentiated, 2= moderately differentiated, 3=poorly differentiated

**Table 2.** Univariate analysis of parameters for disease free survival and overall survival

Parameters	HR	p value	HR	p value
Menopause (yes vs no)	0.53	0.12	0.45	0.18
Surgery (mastectomy vs breast conserving surgery)	1.67	0.30	4.55	0.15
Histology (ductal carcinoma vs others*)	1.43	0.63	1.58	0.66
Tumor size (>T1 vs ≤ T1)	3.00	0.04	1.59	0.55
Lymph nodes (pos vs neg)	1.69	0.24	5.93	0.03
Stage (I,II vs III)	0.49	0.05	0.14	0.01
Estrogen receptor (pos vs neg)	0.79	0.40	0.95	0.93
Progesterone receptor (pos vs neg)	0.67	0.35	0.30	0.12
Hormone receptor status (pos vs neg)	0.68	0.33	0.89	0.84
Grade (III vs II/I)	2.05	0.11	4.00	0.04
NLR				
Low (≤2.38) vs high (>2.38)	0.74	0.46	1.19	0.77
PLR				
Low (≤161.28) vs high (>161.28)	0.89	0.76	1.88	0.30

HR: hazard ratio, \*mixed tumors, medullary tumors

**Table 3.** Disease free survival and overall survival according to NLR and PLR groups

	DFS (months)	95 CI%	p value	OS (months)	95 CI%	p value
NLR						
Low NLR: ≤2.38	68.3	60.4-76.2	0.45	72.7	65.1-80.3	0.77
High NLR: >2.38	59.7	50.7-68.6		76.7	69.9-83.6	
PLR						
Low PLR: ≤161.28	62.6	54.9-69.8	0.76	65.1	57.8-72.4	0.29
High PLR: >161.28	65.9	56.6-75.2		78.6	73.5-83.8	

### Disease free survival and overall survival according to NLR and PLR groups

During follow up, the rate of relapse was 12.6% in the low NLR group, 16.2% in the high NLR group, 12.6% in the low PLR group and 15.8% in the high PLR group. Although median DFS was shorter in the high NLR group vs the low NLR group, the difference was not statistically significant (Figure 1). No statistically significant difference between the high and low PLR groups with respect to both median DFS and median OS was noticed (Table 3).

## Discussion

To our knowledge, this is the first study in the literature to evaluate the prognostic role of both NLR and PLR in HER2-positive early stage breast cancer patients receiving adjuvant trastuzumab. In none of the studies on early stage HER2-positive patients receiving adjuvant trastuzumab was the role of neutrophils, lymphocytes, and thrombocytes taken into account. In the present study, DFS was found to be shorter in patients with high

NLR values. However, no significant relation was found between PLR and DFS or OS.

The relationship between inflammation and cancer is quite important. It is accepted that inflammation in the microenvironment of the tumor facilitates tumor development, invasion and metastasis [9,33]. Unfavorable impact of cancer-associated inflammation on cancer prognosis has been demonstrated by several authors [12,34]. Understanding of a cause-effect relationship between inflammation and cancer is important for the diagnosis and treatment of cancer.

Tumor cells produce many cytokines and chemokines attracting leukocytes. The inflammatory component of a neoplasm may contain many subtypes such as neutrophils, macrophages, and dendritic cells. All of these cells produce cytotoxic mediators that exert a strong effect on tumor development, such as tumor necrosis factor-alpha (TNF- $\alpha$ ), and interleukin-6 [13,35]. In early neoplastic process, inflammatory cells act as strong tumor promoters, producing an environment suitable for tumor growth, stimulating angiogenesis and facilitating genomic instability [9]. Peripheral

neutrophils or neutrophils in the tumor microenvironment produce proangiogenic factors, including vascular endothelial growth factor (VEGF), which stimulate tumor growth [26,27,36]. On the other hand, inflammatory responses may also be antitumoral. However, in cancer patients, inflammatory response is mostly defective [9].

NLR and PLR are easily available simple markers using white blood cell count and platelet numbers and indicate subclinical inflammation. Absolute neutrophil and lymphocyte numbers may be influenced from various physiological, pathological and physical factors. However, NLR has been shown to remain more stable than other leukocyte subtypes [34]. Recent findings indicate that neutrophils play a part in cancer-associated inflammation and have prognostic importance in human cancers [37]. High neutrophil numbers have been demonstrated to be an independent prognostic marker for cancer recurrence and survival (including, gastric cancer, metastatic renal cell carcinoma, metastatic melanoma, and advanced non-small cell lung cancer) [10,13,38]. In order to explain the complex relationship between high NLR and poor prognosis, some suggestions have been put forward [39]. Neutrophils may inhibit the immune system and suppress cytolytic activity of lymphocytes, natural killer cells, and activated T- cells [40]. Paraneoplastic production of myeloid growth factors by cancer is also among the causes of neutrophilia [13]. Tumor-associated neutrophils lead to release of fibroblast growth factor and migration of endothelial cells via enzymatic effect, inducing remodeling of the extracellular matrix. They inhibit the apoptosis of tumor cells via nuclear factor (NF)- $\kappa$ B activation. These events culminate in the enhancement of angiogenesis, tumor growth and progression to a metastatic phenotype [41].

Lymphocytes also have an effect on tumor growth as one of the most important components of the immune system. Tumor infiltrating lymphocytes have been shown to infiltrate tumor mass in melanoma, colorectal cancer, and ovarian cancer, and to decrease tumor recurrence and improve prognosis [42,44]. It is suggested that host cell mediated immunity remains influential on the destruction of residual tumor cells and micro-metastases [45].

Platelets play an important role in cancer progression, promoting cancer growth by enhancing angiogenesis mediated by cytokines [46]. When tissue damage arises, white blood cells and platelets migrate to the damaged region via the

venous system. Thrombocytes secrete platelet-derived growth factor (PDGF), PF4, TGF- $\beta$ , VEGF, and thrombospondin-1 [15-17]. In cancer patients there is an increase in VEGF-A content of thrombocytes and in patients with breast cancer there is a direct correlation between serum VEGF level and the number of thrombocytes in circulation [47].

In the present study, it was established that in breast cancer patients receiving adjuvant trastuzumab, high NLR values were associated with shorter survival. However, no relation was found between PLR and DFS or OS. However, in the univariate analysis, larger tumor size and stage III were found to be associated with DFS, and lymph node positivity, grade III and stage III with OS. In previous adjuvant trastuzumab studies, a tumor size over 2cm, lymph node positivity, and ER positivity were found to be predictive of DFS [30-32] but in these studies the prognostic effect of NLR and PLR had not been evaluated.

High preoperative NLR was reported to be associated with poor prognosis in women with breast cancer [48]. In a previous study on breast cancer NLR cutoff value of 3.3 was reported to have prognostic significance [23]. In the same study, it was established that patients with high NLR had larger tumors and were at more advanced stages. Our NLR cutoff value (2.38) was lower compared to the previous study, as Asians may have lower number of neutrophils, and the higher number of lymphocytes prognostic cutoff value of NLR has been reported to vary with ethnic origins [49].

In a study carried out in our country with 1527 breast cancer patients, high NLR (  $NLR \geq 4$  ) was shown to be an independent predictive factor for both DFS and OS. In that study, lymph node positivity, tumor size, HER2 positivity, the presence of distant metastasis and advanced stage was found to be associated with high NLR whilst ER and PR positivity was found to be associated with low NLR [24]. In the present study, in high PLR and high NLR groups, mean age was found to be lower, and tumor size larger. HER2-positive status of all of our patients and lower mean age may explain this.

In a previous study [23], it was reported that high NLR is predictive of short-term and long-term OS and DFS. High NLR was found to have higher predictive value than other clinicopathological factors such as large tumor size, microvascular and lymphocytic invasion and lymph node involvement [38]. In a recent study with operable breast cancer patients, especially lumi-

nal A subtype, it was reported that elevated pretreatment NLR was associated with shorter breast cancer-specific survival, but the authors did not examine the causes of elevated NLR either [25]. In another study, it was reported that neutrophil-derived oncostatin M induces human breast cancer cells to secrete VEGF and increases the detachment and invasiveness of human breast cancer cells [50].

It is known that HER2-positive tumors have worse biology in comparison to luminal A subtype of triple-negative tumors. It has been suggested that luminal A tumors can be influenced more strongly from the tumor microenvironment than HER2-positive and triple-negative tumors [51-53]. In a large prospective study, evaluating hormonal treatment and its effect on breast cancer, it was demonstrated that luminal A subtypes are influenced from the microenvironment to a larger degree than other subtypes [54]. In another study on ER/PR positive and HER2-negative breast cancer patients, it was reported that in cases with lower pathological response to neoadjuvant treatment, high NLR was associated with shorter RFS and OS [55].

Of our patients, 53.5% were hormone receptor-positive, which may have impacted our results. However, in the present study, among the patients who were HER2-positive and hormone receptor-positive, the number of those with high NLR and PLR was high. No significant difference was found between hormone receptor negative and positive groups with respect to NLR and PLR.

NLR may predict the results of chemotherapy in cancer patients. In 349 patients with metastatic colorectal cancer, the relation between palliative chemotherapy outcome and NLR was evaluated, and high NLR was found to have a significant effect ( $p=0.002$ ). Moreover, following a course of chemotherapy, significant improvement was seen in progression free survival (PFS) in patients whose NLR has returned to normal after a cycle of chemotherapy ( $p=0.012$ ) [56]. In gastric cancer patients, significant relationship was found between pretreatment high NLR ( $NLR>3.79$ ) and low NLR ( $NLR\leq 3.79$ ) and 5-year OS (44 vs 12.2%,  $p<0.01$ ) [10]. In metastatic renal cancer patients receiving sunitinib, a relationship was shown between NLR and OS and PFS [11]. Pretreatment NLR and PLR were reported to have prognostic significance in OS in patients with NSCLC where NLR was shown to be an independent predictor of response to

chemoradiotherapy while PLR was not [14].

More recent evidence established that PLR was closely associated with clinical outcome in patients with ovarian cancer [18,19]. It was also reported in the same study that in patients with epithelial ovarian cancer, PLR was associated with shorter survival DFS and OS. Nevertheless, statistically significant effect of NLR could not be demonstrated [18]. It was also stated that, in ovarian cancer, thrombocyte aggregation, and active thrombocytes can play an important role in tumor survival by increasing tumor cell invasion capacity [57]. In a study [20] with patients with squamous cell oesophageal cancer, preoperative NLR (high  $NLR \geq 3.5$ ) and PLR (high  $PLR \geq 150$ ) were found to have an impact on OS, and PLR was found to be more superior predictive factor than NLR.

Its retrospective nature, small sample size, relatively short follow-up in some patients and small number of patients with recurrence may be cited as the limitations of the present study. Its retrospective design may have led to bias in data selection and analysis. Moreover, patients were not distributed evenly according to clinicopathological factors, and there was not adequate information on the relation of these factors with NLR, which also might have led to bias. However, NLR is a cheap and readily available test that can be used in daily practice.

As it can be seen in different studies, NLR and PLR were shown to exert different effects on DFS, OS and response to treatment. Based on these findings, correlation of high NLR with prognosis should be supported with further studies. There is an ongoing process in the tumor microenvironment, and we are not aware of its details. As reported in previous studies, are HER2-positive tumors influenced from tumor environment to a lower degree than hormone receptor positive tumors or are there other mediating factors in tumor microenvironment? This question still remains to be answered.

In conclusion, the present study established that high pretreatment NLR may be influential on DFS in HER2-positive early breast cancer patients receiving adjuvant trastuzumab. Further studies are required to explain the effect and mechanism of pretreatment NLR and PLR in HER2-positive tumors. Prospective studies with larger patient numbers will make it possible to obtain more reliable results.

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