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Research Article

Predictive Value of Lymphocyte-Related Blood Parameters of before Ado-Trastuzumab Emtansin Treatment in Breast Cancer

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Abstract

Objective: Ado-Trastuzumab Emtansine (TDM1) has been used in the treatment of HER2 metastatic breast cancer after FDA approval as an antibody-drug conjugate. Some markers may be predictive for clinician in the management of this TDM1 therapy. There is a need for simple and cost-effective markers to show the treatment response. The primary aim of this study is to establish a correlation between progression free survival and pre-treatment hematological inflammatory parameters [absolute lymphocyte count (ALC), neutrophil-to-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR)] in advance breast cancer.

Methods: Forty-one female patients with metastatic HER-2 positive breast cancer who received TDM-1 between 2016 and 2021 were evaluated retrospectively. NLR and PLR were calculated with the values of neutrophils, lymphocytes, and trombocytes in complete blood count at the time of diagnosis. The cut off values of NLR and PLR were determined using receiver operating characteristic (ROC) curve analysis. Overall survival (OS) and progression free survival (PFS) associated with prior treatment NLR, PLR, ALC were performed by Kaplan-Meier method.

Results: Median age was 49.5 years years (26-76).Patients divided into 2 groups according to NLR cut-off and TLR cut-off values as NLR/TLR high and low groups. The cut-off values of NLR and TLR were 144.0, and 2.74, respectively. ALC divided into 2 groups. There was no significant difference in OS between NLR ,PLR,ALC (high/low) groups. (p=0.04, 0.15, 0.53 respectively). There was only significant difference in PFS (high/low) groups.(p=0.81, 0.99, 0.96 respectively).

Conclusions: The predictive value of pre-treatment only NLR on the therapeutic potential in patients with metastatic breast cancer treated with TDM1.

Keywords: Ado-Trastuzumab Emtansine (TDM1), Progression Free Survival (PFS), Absolute lypphocyte Count (ALC), Neutrophil-to-lymphocyte Ratio (NLR), Platelet-lymphocyte Ratio (PLR).

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HER2 is a member of the human epidermal growth factor receptor family. It is a transmembrane receptor with tyrosine kinase activity and is normally involved in cell growth and proliferation. If HER2 overexpression oc-curs due to HER2 gene amplification, malignant transformation of cells may occur as a result. HER2 overex-pression is detected in 30% of breast cancer cases.[1] HER2 overexpression is generally associated with a more aggressive tumor phenotype, poor prognosis, and shortened relapse time.[2] However, thanks to the drugs de-veloped for HER2, there

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have been important developments in the treatment of HER-2 positive breast cancer, in recent years. For patients with metastatic HER2 positive breast cancer, the 5-year overall survival was 8.1% in 1995, compared to 34.0% in 2015. While the median survival was 26 months in 1995, it reached 50.8 months in 2015.[3]

Ado-trastuzumab emtansine is an antibody-drug conjugate consisting of trastuzumab, a humanized monoclo-nal antibody covalently linked to the cytotoxic agent DM1.[3, 4] Trastuzumab alone inhibits the growth of cancer cells by binding to the HER2 receptor, whereas trastuzumab emtansine enters cells via the receptor, catab-olizes in lysosomes where DM1-containing catabolites are released, and then binds to tubulin to cause mitotic arrest and cell death. Binding to HER2, trastuzumab prevents homo-dimerization or heterodimerization (HER2/HER3) of its receptor, ultimately inhibiting the activation of the MAPK and PI3K/AKT cellular signal-ing pathways. Since the monoclonal antibody targets HER2 and HER2 is only overexpressed in cancer cells, the conjugate cytotoxic agent delivers DM1 specifically to tumor cells.[5] The conjugate is abbreviated as T-DM1.In the EMILIA clinical trial,[6] in women with trastuzumab-resistant, advanced HER2-positive breast cancer, TDM-1 improved median overall survival by 5.8 months (30.9 versus 25.1 months) compared to the combination of lapatinib and capecitabine. Based on this, the US Food and Drug Administration (FDA) ap-proved marketing on February 22, 2013.[7]

Immune responses against cancer cells may involve a balance between tumor inhibitory mechanisms induced by intrinsic immunity and the ability of tumor cells to evade immune surveillance.[8] During the cancer im-munomodulation stages (ie, elimination, equilibrium, and escape), activated effector cytotoxic T lymphocytes can migrate to the tumor and infiltrate the tumor microenvironment.[9] It has been reported that circulating pe-ripheral lymphocytes and neutrophils can migrate towards a tumor site in a directed manner along the humoral factors, such as a chemoattractant.[9] A recent study demonstrated that circulating tumor-specific T lymphocytes related to neoantigens can be isolated from the peripheral blood of patients with melanoma.[10] These data may indicate that peripheral blood-based parameters (PBBPs) reflect a local immune reaction against cancer cells. In line with such a mechanism, the absolute lymphocyte count (ALC), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) are reported to be immune-related prognostic factors for patients with malig-nancy.[11,12] In addition, relationships between low levels of NLR and improved response to neoadjuvant chemotherapy have been reported for patients with breast cancer.[13-15] Therefore, PBBPs seem to affect treat-ment efficacy for Advance Breast Cancer (ABC) treated with chemotherapy and anti-HER2 therapy; However, trastuzumab and pertuzumab have been evaluated in studies on this subject[16] and it has not answered the question of whether it can be used as an indicator for the response of TDM1 treatment.

In this study, it was primarily aimed to investigate whether PBBPs are associated with progression-free survival (PFS) in HER2-positive ABC patients treated with TDM-1. As a secondary aim, it was investigated whether these hematological parameters were correlated with the prognosis.

Methods

Patients

Patients with HER-2 positive metastatic breast cancer who received treatment at multiple centers, between January 2016- April 2021, were retrospectively evaluated. Medical records of the hospitals were reviewed in terms of age, primary breast surgery, ECOG performance status, number of metastatic sites, hormonal status, HER-2 positive was 3+ IHC testing or it was 2+ on IHC testing, confirmed by fluorescence in in situ hybridiza-tion (FISH). An addition before TDM-1 received treatment agents, hematological parameters including lympho-cyte, thrombocyte, neutrophil (prior treatment), last visit date, and date of death if the patient was deceased were saved to the SPSS program and all data were included in the study. The exclusion criteria was co-morbidities that may alter peripheral blood count (presence of rheumatological diseases, existing chronic liver or chronic renal diseases) and drug use (antibiotic use within the last week before TDM-1, corticosteroid use). All the pro-cedures were conducted according to the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of Celal Bayar University, School of Medicine (number E.78438; Manisa, Turkey).

Drug Dosing Schedule

Ado-trastuzumab emtansine was used in the treatment of HER-2 positive ABC in the second and next lines as mentioned previously. It was administered according to a standard procedure 3 mg /kg intravenously over 30 min every 3 weeks. No dose reduction was performed.

NLR and PLR

NLR was defined as the ratio of absolute neutrophil and lymphocyte count within one weeks before initia-tion of TDM-1 treatment and the end of the 3rd cycle of TDM-1 treatment. PLR was defined as the ratio of thrombocyte and lymphocyte count within one weeks before the initiation of TDM-1 treatment

NLR was divided into two groups according to the cut-off points ≥2.74 or <2.74 as NLR high and low (area under the curve: 0.648, specificity: 0.53, sensitivity: 0.67).

PLR was divided into two groups based on the cut-off points (≥144.0 or <144.0) as PLR high and low (area under the curve: 0.704, specificity: 0.60, sensitivity: 0.82). The cut off values of NLR and PLR were deter-mined using ROC curve analysis.

Absolute Lymphocyte Counts

The cut off value for ALC were st at 1000 or 1500 cells/ µL. The cutoff value for ALC was based on the previous study. [16] The value of ALC in terms of PFS was evaluated using a forest plot for each subgroup, dichotomized as <1000, 1000-1500 and ≥1500 cells/µL.

Statistical Analysis

Overall survival (OS) and progression free survival (PFS) associated with prior treatment NLR, TLR, ALC were performed by Kaplan-Meier method and log-rank test was used for comparison of survival distribution among groups. Patient age, primary breast surgery, ECOG performance status, number of metastatic sites, hormonal status, HER-2 status (immunochemical or FİSH), before TDM-1 received treatment agents compared by Chi-square, Fisher's exact and Mann-Whitney U tests as appropriate. Univariate analyzes were performed via a Cox-proportional hazards model. SPSS 22.0 (SPSS Inc., Chicago, IL, USA) software was used in all statistical analyses. A p value of <0.05 was considered as significant.

Results

General Characteristics

A total of 41 patients were included in this study. The median age was 49.52±11.9 years (29-76 years). De-mographic features of the patients including age, primary breast surgery, ECOG performance status, number of metastatic sites, hormonal status, HER-2 positive (immunochemical or FİSH), before TDM-1 received treat-ment agents, hematological parameters including lymphocyte, thrombocyte, neutrophil (prior treatment), last visit date, and date of death if the patient was deceased were given in table 1.

Survival Analysis

Patients were divided into two groups according to NLR as NLR-low and NLR-high. Sev-enteen (40.4%) patients were in NLR-low group and 25 (59.6%) patients were in NLR-high

Table 1. Demographic and clinical features of the patients and data on treatment

	All patient (n=42) (%)	PFS (mounth ±SD)	p	0S (mounth±SD)	p
Age	49,5 ±11,9				
ECOG 0	13 (%31)	13.30±9.43	p=0.168	41.5±10.7	
ECOG 1	23 (%54.8)	8.62±7.30		48.8±17.8	p=0.19
ECOG 2	6 (%14.3)	15.7±15.9		39.4±19.7	
Primary surgery					
No	8 (%19)	4.2±1.95	p=0.005	30.03±2.42	p=0.12
Yes	34 (%80)	12.71±10.07		56.38±24.2	
Number of metastat-ic site					
1	7 (%16)	13.5±3.1	p=0.273	*	p=0,49
2	17 (%40)	11.7±2.9		46.6±8.1	
3	10 (%64)	7.9±2.1		52.7±11.0	
≥4	8 (%19)	13.8±3.5		70.9±14.4	
HR statement					
Negative	12 (%28.6)	11.2 ±8.78	p=0.1	40.07±7.05	p=0.31
Positive	30 (%71.4)	11.04 ±10.1		58.01±28.03	
Treatment before TDM1					
Pertuzumab	8 (%19)	12.7±1.72	p=0.006	**	**
Lapatinib	17 (%40)	11.3±2.2	p=0.54	53.7±7.19	p=0.50
TDM1 which line					
2. line	18 (%42)	9.02±1.64	p=0.606	39.5±11.3	p=0.404
3. line	11 (%26)	13.1±4.16		53.2±25.6	
≥ 4. line	13(%30)	12.2±2.48		58.6±28.6	

* The OS omitted as there was only one death in patients with one of metastatic site; ** The OS could not be calculated because there were no deaths in patients receiving Per-tuzumab as first-line therapy.

Table 2. Demografik feature of patients according to NLR low vs high and PLR low and high

	All patients	NLR low	NLR high	p	PLR low	PLR high	p
Age	48.5 (29-76)	47.5 (29-76)	50 (30-76)	0.2	54.5 (29-67)	48.5 (30-76)	0.32
ER +	30 (71.4%)	13 (43.3%)	17 (56.7%)	0,73	11 (36.7%)	19 (63.3%)	0,72
ER -	12 (28.6%)	4 (33.3%)	8 (66.7%)		3 (25%)	9 (75%)	
PR +	19 (45.2%)	8 (42.1%)	11 (57.9%)	1	8 (42.1%)	11 (57.9%)	0,34
PR -	23 (54.8%)	9 (39.1%)	14 (60.9%)		6 (26.1%)	17 (73.9%)	
Surgery							
yes	34 (81%)	14 (41.2%)	20 (58.8%)	1	10 (29.4%)	24 (70.6%)	0,41
No	8 (19%)	3 (37.5%)	5 (62.5%)		4 (50%)	4 (50%)	
Metastatic site							
<=2	24 (57.1%)	8 (33.3%)	16 (67.7%)	0,35	9 (37.5%)	15 (62.5%)	0,72
>2	18 (42.9%)	9 (50%)	9 (50%)		5 (27.8%)	13 (72.2%)	
ECOG-0	13 (31%)	5 (38.5%)	8 (61.5%)	0,87	4 (30.8%)	9 (69.2%)	0,57
ECOG-1	23 (54.8%)	9 (39.1%)	14 (60.9%)		9 (39.1%)	14 (60.9%)	
ECOG-2	6 (14.3%)	3 (50%)	3 (50%)		1 (16.7%)	5 (83.3%)	

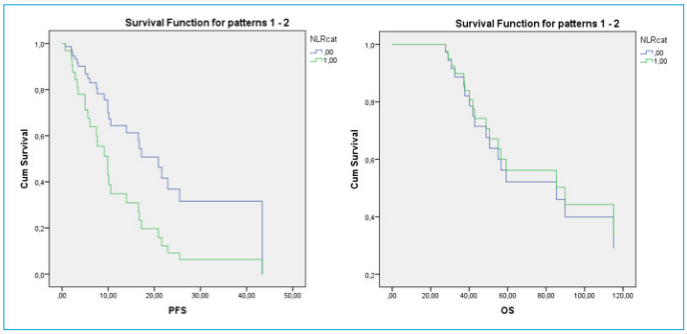

Figure 1. Association between NLR and survival (PFS /OS).

group. Median overall survival was similar in NLR-low and NLR-high groups, 54.+ 7.14 months (95% CI 39.30-69.61) vs 63.70+8.85 months (95% CI 45.41-81.9); p=0.809], respectively. Median PFS was 13.91+2.79 months(95% CI 7.98-19.85) in NLR-low group and 9.18+1.57months (95% CI 5.93-12.42) in NLR-high group, p=0.82 (Table 2, Fig. 1).

Additionally, patients were grouped according to PLR as PLR-low and PLR- high. There were 14 (33.3%) patients in PLR-low group and 28 (66.6%) patients in PLR-high group. There was no difference in terms of OS and PFS between groups PLR-low and PLR-high. Median OS in PLR-low and PLR-high groups were 51.68+12.75months (95% CI 11.08-92.2) vs 51.81+6.87 months (95% CI 36.84-66.78); p=1], respectively. Median PFS in PLR-low and PLR-high groups were 10.35+3.11months (95% CI 3.63-17.07) vs 11.47+1.65months (95% CI 8.07-14.86); p=0.77], respectively (Table 2, Fig. 2).

Patients were divided into three groups according to ALC as <1000, 1000-1500 and ≥1500 cells/μL. Six-teen (38%) patients were in ALC-low group; 13 (30%) patients were in ALC middle group and 13 (30%) patients were in ALC-high group. Although the ALC-high group curve appears higher in others, there was no difference between groups in PFS (p=0.53) and OS (Fig. 3).

Univariate analysis revealed that NLR ,PLR, ALC had no effect on the OS but only NLR had effect on PFS in patients with treated with TDM-1 (p=0,04) (Table 4).

Discussion

The prognostic significance of the NLR and PLR have been researched in many tumors.[17,18] Previous studies claimed

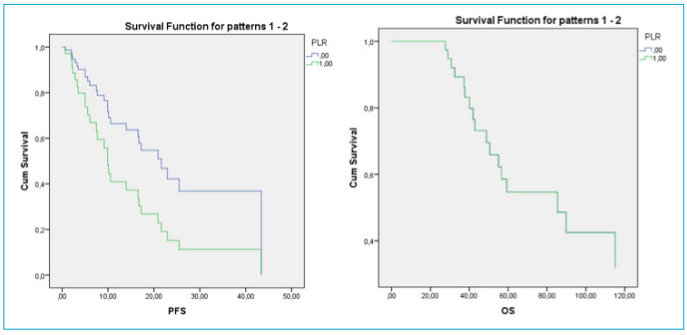

Figure 2. Association between PLR and survival (PFS /OS).

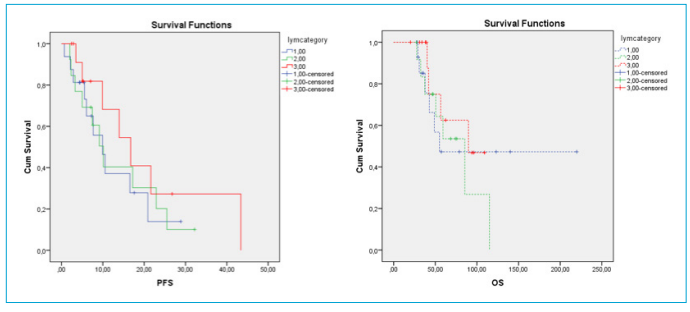

Figure 3. Association between ALC and survival (PFS /OS).

Table 3. NLR low vs high and PLR low vs high in univariate analysis

	PFS		OS	
	Univariate analysis, HR (95%CI)	**p**	**Univariate analysis, HR (95%CI)**	**p**
NLR low vs high	2,4 (1,03-5,58)	0,04	0,89 (0,34-2,35)	0,81
PLR low vs high	2,18 (0,75-6,36)	0,15	0,99 (0,32-3,15)	0,99
ALC low vs high	0,77 (0,35-1,72)	0,53	1,03 (0,37-2,84)	0,96

that increased systemic inflammatory markers such as NLR and PLR are associated with poor prognosis in metastatic breast cancer.[18] However, the effect of these markers on chemotherapy efficiency is not well known in HER-2 metastatic breast cancer. In our study, we analyzed the association between pre-treatment NLR, PLR levels and ALC with TDM-1 treatment response in patients with HER-2 positive metastatic breast cancer.

Previous studies used similar NLR cut-offs, such as 2 or 5, to differentiate patients into low- and high-risk groups.[19-20] However, we defined a more accurate cut-off level using the ROC curve analysis. We used the 2.74 value to categorize our patients into NLR low-risk group (<2.74) and NLR high-risk group (≥2.74). In our cohort, patients with low NLR did not have better PFS (PFS; 7.1 months vs 9.9 months). But patients with high NLR had worse OS (40.1 months vs 48.8 months). In the subgroup analysis, patients with NLR-low consistently had longer PFS compared to those with NLR-high irrespective of the number of prior chemo-therapy regimens, prior trastuzumab, visceral metastasis, estrogen receptor status, and human epidermal growth factor receptor 2 (HER2) score.

Likewise, reports about the association of PLR with survival outcomes used PLR cut-offs, such as 150 or 200, that usually differentiate patients into low- and high-risk groups.[21-22] We used the 144.0 value, estimated with ROC curve analysis, to categorize patients into a PLR low-risk group (<144.0) and PLR high-risk group (≥144.0). OS and PFS were similar in PLR low-risk group and high-risk group (PFS; 7.1 months vs 8.4 months, OS; 39.8 months vs 48.8 months).

In studies with ALC, lymphocytes were grouped as below 1000 and above 1500.[15] When we divided this cohort as below 1000, between 1000-1500 and over 1500, statistically signifi-cant results were not found in PFS and OS. (respectively PFS; 12.3 months, 13.4 months, 21.08 month p=0,53). Although the ALC low (red line) in the PFS curve was different in the others, statistical significance was not obtained due to the number of patients.

Hematologic parameters, excluding NLR, were not significantly associated with the progno-sis of HER2-positive breast cancer.[23] But Ulas et al. was reported that in patients with early breast cancer receiving adjuvant trastuzumab, there was no significant association between NLR levels and DFS or OS.[24] And another study was reported previously identified the useful-ness of the NLR in HER2-negative primary advanced and recurrent breast cancers treated with eribulin but not with nab-paclitaxel.[16] Similarly, Vernieri et al. showed that high NLR was significantly associated with lower PFS in Triple negative breast cancers treated with platinum-containing chemotherapy but this association was not significant in the ER-positive/HER2-negative cancers.[25] These data suggest the predictive value of NLR for a part of treatment efficacy induced by chemotherapy in ABC. Thus, NLR could serve not only as a prognostic but also as a predictive indicator for TN breast cancers, but the significance of NLR still remains unclear in HER2-positive breast cancers.

Lymphocyte counts may reflect an immune reaction or potential immunity against cancer cells. On the contrary, cytokines and chemokines produced by neutrophils play a key role in promoting tumor progression.[26,27] Thus, the NLR, the ratio of these two factors, is consid-ered an indirect indicator for immune reaction, i.e., a low NLR indicates high immunity against cancer cells. This hypothesis may be supported by the report that demonstrated significant asso-ciations between the NLR and serum cytokines related to inflammation, including interleukin-6, -8, and -2Rα; hepatocyte growth factor; macrophage-colony stimulating factor; and vascular ep-idermal growth factor A; in colorectal cancer.[28] In addition, NLR levels were positively as-sociated with the concentration of myeloid-derived suppressor cells in peripheral blood but neg-atively associated with interferon-γ in breast cancers.[29] Based on these reports, a high NLR may represent an immune suppressive state in the tumor microenvironment. Therefore, we speculate that T-DM1 efficacy may be higher in patients with a low NLR, which reflects lower immune suppression and immune induction related to T-DM1 can be expected. Modulation of the immune reaction by T-DM1 is recognized in the present study by a decrease in the NLR and increased lymphocyte counts after one cycle of treatment. Although neutrophils appeared to be suppressed due to chemotherapy, a signifi-

cant increase in lymphocytes after treatment with T-DM1 may indicate direct or indirect immune activation by this drug. Since a significant increase in lymphocytes was recognized in the NLR-low but not in the NLR-high group, we estimated that the induction of lymphocyte-based immunity was possibly inhibited under a high neutrophil concentration. The observation by Müller P et al. that T-DM1 elicits antitumor immunity might be in line with our results.[30]

Kazuhiro Araki et. al. aimed to evaluate whether peripheral blood-based parameters could have predictive value in HER2-positive advanced breast cancer treated with pertuzumab and trastuzumab combined with eribulin or nab-paclitaxel (nab-PTX). They identified ALC as a predictive factor for PFS in patients with HER2-positive advanced breast cancer . Additionally, low ALC at baseline was significantly associated with improved PFS in HER2-positive advanced breast cancer treated with either ERI or Nab-PTX in combination with nab-PT X.[15] However, those investigated in our study are not predictive or prognostic factors associated with ALC in HER-2 positive advanced breast cancer treated with TDM-1.

Considering the secondary contributions of the study, it is seen that patients who underwent primary breast surgery and pertuzumab treatment before TDM-1 contributed to progression-free survival, and even in patients who received pertuzumab, there was a significant improvement in OS.

The major limitations of our study are its retrospective nature, small sample size single-arm turkey-based cohort design. Medical records of the hospitals were reviewed in terms of age, metastasis status as de novo metastasis or at time of relapse, number of metastatic sites, hormo-nal status, HER-2 positive However, it provides a real-life data about the prognostic signifi-cance of NLR, PLR and ALC that assessed just before the TDM-1 treatment and after the TDM-1treatment , in patients with breast cancer. The validity of the applied NLR and PLR cut-off, the median value and superior cut-off, which is an absolute value, should be investigated in a future prospective study with a larger sample size.

Conclusion

In our study, we could demonstrate the predictive value of pre-treatment only NLR on the ther-apeutic potential in patients with metastatic breast cancer treated with TDM1. However, the same results were not obtained for other markers. Also we suggest that these markers have lim-ited prognostic value in patients treated with TDM-1.

As the most important result of our study, NLR was found to be the most reliable hemato-logical inflammatory marker for HER-2 positive metastatic breast cancer, and it can be claimed to predict TDM1 treatment response.

Disclosures

Ethics Committee Approval: All the pro-cedures were conducted according to the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of Celal Bayar University, School of Medicine (number E.78438; Manisa, Turkey).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – G.B.; Desing – A.O.; Supervision – A.P.E.; Materials – A.O.; Data collection – F.E., S.Y.; Analysis and interpretation – A.O., S.Y.; Literature search – C.Y.; Writing – G.B.; Critical Review – S.Y., A.P.E.

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