The prognostic significance of pretreatment neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in patients with resectable non-metastatic breast cancer

Mohamed El-Shebiney[1], Alaa Maria[1], Muhammed T. Abdelghafar[2]

[1]Clinical Oncology, Faculty of Medicine, Tanta University, Egypt
[2] Clinical Pathology Departments, Faculty of Medicine, Tanta University, Egypt
alaamaria1@hotmail.com

Abstract: Purpose: The objective of this trial is to explore the correlation between pretreatment neutrophil-to-lymphocyte ratio (NLR) and a platelet-to-lymphocyte ratio (PLR) and the disease-free survival (DFS) of patients with early resectable, non-metastatic breast cancer (BC) treated with neoadjuvant chemotherapy (NAC). **Patients and methods:** Receiver operating characteristic (ROC) curve analysis was utilized to determine an ideal cut-off value for NLR and PLR to discriminate between patients' DFS. Accordingly, 134 BC patients were classified into low and high NLR and PLR groups, respectively. **Results:** The ROC curve analysis determined 2.2 and 180 as optimal cut-off values for NLR and PLR respectively. On univariate analysis, both high NLR and PLR significantly correlated with poor DFS. On multivariate analysis, the significant prognostic value of high NLR continued (CI: 1.7-5.9, $p<0.001$), but not for PLR (CI: 0.5-1.6, $p=0.595$). Additionally, LNs involvement and high Ki-67 level significantly affect the DFS. The overall clinical response rate (RR) significantly correlated with the lower value of both NLR and PLR ($p<0.001$ for both). **Conclusion:** The high NLR significantly correlated with poor DFS in patients with early non-metastatic BC treated with NAC, but PLR is not. As NLR is a clinical marker that can be easily applied and its high value was correlated with poor prognosis of early BC patients, NLR might be a potential predictor in patients' outcome to assist in treatment decisions.

1. Introduction

Considering the connection between cancer and inflammation is important for cancer diagnosis and treatment [1]. The inflammatory cells in the early neoplastic process render the environment more suitable for tumor growth, acting as strong tumor promoters, enhancing genomic instability, and stimulating angiogenesis. Oppositely, although the inflammatory response is most defective in cancer patients, it may have some antitumor mechanisms [2].

Neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) indicate subclinical inflammation and are simple daily practice prognostic markers. Several pathological, physiological, and physical factors may influence the absolute lymphocyte and neutrophil counts. However, NLR is the most stable form among all leukocyte subtypes [3].

In various types of metastatic cancer (e.g., lung cancer, gastric cancer, melanoma, and renal cell carcinoma) an elevated neutrophil number was considered to be a poor prognostic marker for survival and cancer recurrence [4]. A lot of suggestions to explain the complex correlation between poor cancer prognosis and high NLR were reported. Neutrophils suppress activated T-cells, natural killer (NK) cells and cytolytic activity of lymphocytes with consequent inhibition of the immune system [5]. In addition, tumor-associated neutrophilia enhance the release of fibroblast growth factor and via enzymatic effect causes migration of endothelial cells, inducing remodeling of the extracellular matrix. Also, neutrophils activate nuclear factor (NF)-kB leading to inhibition of the tumor cells apoptosis [6]. Interaction of neutrophils with cancer cells can produce cytokines for instance vascular endothelial growth factor (VEGF) [7]. All of these occasions lead to augmentation of tumor growth, tumor angiogenesis, and progression to a metastatic phenotype.

Platelets also share in cancer progression. Via the release of metalloproteases, platelets can support cancer cell extravasations. Platelets enhance tumor growth at the metastatic site and tumor angiogenesis via the release of angiogenic growth factors e.g. VEGF and platelet-derived growth factor (PDGF), which enable metastatic spread [7]. Platelets also protect the circulating tumor cells (CTC) from natural killer (NK) T-cell-mediated cytolysis [8].

The systemic inflammatory response is usually associated with the release of many pro-inflammatory

mediators such as interleukin (IL)-1, IL-3, and IL-6 leading to thrombocytosis through stimulation of the megakaryocyte proliferation. Consequently, release of platelet-derived pro-angiogenic mediators, platelet aggregation and degranulation are suggested to be important determinants of tumor growth [9]. Some of the immunologic mediators, such as transforming growth factor-b (TGF-b), and IL-10 are released and can result in reduced lymphocyte counts and impairment of lymphocyte function with consequent immunosuppressive effect [10].

Lymphocytes are important components of the immune system that inhibit tumor cell proliferation and metastasis. Tumor-infiltrating lymphocytes (TILs) were documented to infiltrate tumor mass in ovarian cancer, colorectal cancer, and melanoma, and to decrease tumor recurrence and improve prognosis [11].

The NLR and PLR are easily measured, reasonable and practical markers that their prognostic role in breast cancer (BC) had not yet clear. We planned this retrospective study to evaluate the prognostic significance of NLR and PLR hematological markers on the disease-free survival (DFS) in a population of resectable BC patients treated with neoadjuvant chemotherapy (NAC).

2. Patients and Methods

In the present study, we assessed retrospectively female patients with histologically confirmed resectable, early-stage BC diagnosed as stage IIA (except T0, N1, M0), IIB or IIIA who had planned to receive NAC at Clinical Oncology Department, Tanta University hospital during the period between March 2009 and June 2015. The study was approved by the Medical Ethics Committee of the Faculty of Medicine. The clinicopathological and the laboratory data of 134 patients were available for the analyses.

The primary endpoint was an assessment of the significant effect of pretreatment NLR and PLR among other clinicopathological variables on DFS. The secondary endpoints were evaluation the clinical response according to pretreatment NLR & PLR and correlation between intrinsic subtypes and DFS according to pretreatment NLR & PLR.

Eligibility criteria

The eligible patients fulfilled the following criteria: histologically confirmed BC, resectable non-metastatic and non-inflammatory tumors, available report of pretreatment complete blood count (CBC) with differential white blood cell count, and no history of blood disease, renal disease, autoimmune disease, chronic inflammatory disease, steroid treatment, blood transfusion or active bleeding within the last 3 months.

Patient's samples

The absolute cell counts of neutrophils, lymphocytes, and platelet attained before the first cycle of NAC were analyzed using the receiver operating characteristic (ROC) curve analysis to determine an ideal cut-off value of NLR and PLR. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. The PLR was defined as the absolute platelet count divided by the absolute lymphocyte count. All patients were categorized into two groups according to the NLR cut-off value (high ≥2.2 versus low <2.2) and according to the PLR cut-off value (high ≥180 versus low <180).

The clinicopathological data obtained from the patients' medical files included: medical history, age, menopausal status, clinical stage, chemotherapeutic regimen, surgical type, pathological type, tumor diameter, tumor grade, the axillary lymph nodes (LNs) status, lymphovascular invasion (LVI), hormonal receptor (estrogen receptor, ER and progesterone receptor, PR) status, human epidermal growth factor receptor 2 (HER2) expression, Ki-67 values, clinical response, pathological response, time of local or distant failure, and time of death or last follow up. The clinical and pathological TNM stages were determined according to revisions for the 7th edition of the American Joint Committee on Cancer (AJCC) TNM staging system.

Immunohistochemistry (IHC) studies and their interpretation

Sections of formalin-fixed paraffin-embedded block of all patients were stained for IHC study. Estrogen receptors and PR were considered positive for expression when >1% of the cell nuclei stained with the antibody. Human epidermal growth factor receptor 2 (HER2) considered positive for expression when IHC staining was 3+ (uniform, intense membrane staining of >30% of invasive tumor cells) or IHC 2+ that were fluorescent in situ hybridization (FISH) positive. According to the recommendations of the International ki-67 in Breast Cancer Work group, the Ki-67 score was defined as the percentage of the total number of tumor cells with nuclear staining by the antibody [12].

Treatment

Neoadjuvant CT was administered in the form of anthracycline-based regimens (46; 34.3% patients), taxane-based regimens (50; 37.3% patients), and combined anthracycline with taxane-based regimens (38; 28.4% patients). All patients had breast surgery with 85 (63.4%) patients had breast-conserving surgery and 49 (36.6%) patients had modified radical mastectomy. Seventy-six (56.7%) and 129 (96.3%) patients received adjuvant CT and radiotherapy respectively. Adjuvant hormonal therapy was administered to all patients with positive hormonal receptors while 14 out of 24 patients with HER2 overexpression received adjuvant trastuzumab therapy.

Follow up

All patients had a clinical examination, laboratory investigations, and radiological assessment every 3 months throughout the first 3 years and later every 6 months. Suspicious recurrent lesions were assessed pathologically with further diagnostic methods were performed as appropriate.

Statistical analysis

Optimal cut-off values for NLR and PLR were determined by employing the ROC curve analysis. Differences in categorical variables in the 2-way table were analyzed by the chi-square test. The DFS was estimated from the time of NAC to the documentation of the first failure (local or distant), death or last follow-up. Kaplan-Meier method with the log-rank test was used for estimation and comparing the survival rate. Factors affecting the DFS rate were analyzed by the Cox proportional hazards model. All analyses were performed using SPSS software,

version 21.0 and p-value <0.05 considered statistically significant.

3. Results

Table 1 shows the baseline clinicopathological characteristics of 134 early BC patients. All patients were divided by high NLR/PLR and low NLR/PLR ratios. The median age of all patients was 50 years (range 27-68) with 55.2% of patients were menopause. Eighty-four percent of the patients presented with tumor size >2 cm. Grade 2 tumors were the most common representing 53.7% of the patients and 70.1% of the patients had positive LNs. Estrogen receptor positive tumors were detected in 80.6% with HER2 overexpression in 17.9% of the patients and Ki-67 level ≥14% was found in 44.8% of the patients. Luminal subtype (A & B) was the most common intrinsic subtype (80.6%).

Table 1: Baseline clinicopathological characteristics of 134 breast cancer patients

Characteristics	Whole patients No. (%)	NLR			PLR		
		<2.2 (n=70) No. (%)	≥2.2 (n=64) No. (%)	χ2	<180 (n=71) No. (%)	≥180 (n=63) No. (%)	χ2
Age (years)							
Median	50			0.089			0.830
Range	27-68						
Mean±SD	49.5±8.1						
≤50	71 (53.0)	42 (59.2)	29 (40.8)		37 (52.1)	34 (47.9)	
>50	63 (47.0)	28 (44.4)	35 (55.6)		34 (54.0)	29 (46.0)	
Menopausal status							
Menopause	74 (55.2)	34 (45.9)	40 (54.1)	0.105	37 (50.0)	37 (50.0)	0.442
Premenopausal	60 (44.8)	36 (60.0)	24 (40.0)		34 (56.7)	26 (43.3)	
Tumor size							
≤2 cm	21 (15.7)	9 (42.9)	12 (57.1)	0.349	11 (52.4)	10 (47.6)	0.952
>2 cm	113 (84.3)	61 (54.0)	52 (46.0)		60 (53.1)	53 (46.9)	
Histology							
IDC	127 (94.8)	65 (51.2)	62 (48.8)	0.296	67 (52.8)	60 (47.2)	0.821
ILC	7 (5.2)	5 (71.4)	2 (28.6)		4 (57.1)	3 (42.9)	
Tumor grade							
Grade 1	14 (10.4)	12 (85.7)	2 (14.3)	<0.001	11 (78.6)	3 (21.4)	<0.001
Grade 2	72 (53.7)	46 (63.9)	26 (36.1)		47 (65.3)	25 (34.7)	
Grade 3	48 (35.8)	12 (25.0)	36 (75.0)		13 (27.1)	35 (72.9)	
LVI							
Yes	71 (53.0)	24 (33.8)	47 (66.2)	<0.001	27 (38.0)	44 (62.0)	<0.001
No	63 (47.0)	46 (73.0)	17 (27.0)		44 (69.8)	19 (30.2)	
Clinical T-stage							
T1	21 (15.7)	9 (42.9)	12 (57.1)	0.358	11 (52.4)	10 (47.6)	0.860
T2	67 (50.0)	39 (58.2)	28 (41.8)		37 (55.2)	30 (44.8)	
T3	46 (34.3)	22 (47.8)	24 (52.2)		23 (50.0)	23 (50.0)	
Clinical LN status							
N0	40 (29.9)	31 (77.5)	9 (22.5)	<0.001	33 (82.5)	7 (17.5)	<0.001
N1	60 (44.8)	29 (48.3)	31 (51.7)		24 (40.0)	36 (60.0)	
N2	34 (25.3)	10 (29.4)	24 (70.6)		14 (41.2)	20 (58.8)	

AJCC stage							
IIA	38 (28.4)	21 (55.3)	17 (44.7)	<0.001	27 (71.1)	11 (28.9)	0.003
IIB	52 (38.8)	36 (69.2)	16 (30.8)		29 (55.8)	23 (44.2)	
IIIA	44 (32.8)	13 (29.5)	31 (70.5)		15 (34.1)	29 (65.9)	
ER							
Positive	108 (80.6)	58 (53.7)	50 (46.3)	0.489	56 (51.9)	52 (48.1)	0.592
Negative	26 (19.4)	12 (46.2)	14 (53.8)		15 (57.7)	11 (42.3)	
PR							
Positive	98 (73.1)	52 (53.1)	46 (46.9)	0.753	48 (49.0)	50 (51.0)	0.125
Negative	36 (26.9)	18 (50.0)	18 (50.0)		23 (63.9)	13 (36.1)	
HER2 status							
Positive	24 (17.9)	8 (33.3)	16 (66.7)	0.041	11 (45.8)	13 (54.2)	0.438
Negative	110 (82.1)	62 (56.4)	48 (43.6)		60 (54.5)	50 (45.5)	
Ki-67 values							
<14%	74 (55.2)	46 (62.2)	28 (37.8)	0.011	46 (62.2)	28 (37.8)	0.018
≥14%	60 (44.8)	24 (40.0)	36 (60.0)		25 (41.7)	35 (58.3)	
Subtype							
Luminal A	60 (44.8)	38 (63.3)	22 (36.7)	0.128	37 (61.7)	23 (38.3)	0.110
Luminal B	48 (35.8)	20 (41.7)	28 (58.3)		19 (39.6)	29 (60.4)	
HER2 positive	14 (10.4)	7 (50.0)	7 (50.0)		9 (64.3)	5 (35.7)	
Triple negative	12 (9.0)	5 (41.7)	7 (58.3)		6 (50.0)	6 (50.0)	

NLR: Neutrophil/lymphocyte ratio; PLR: Platelet/lymphocyte ratio; IDC: Invasive duct carcinoma; ILC: Invasive lobular carcinoma; LVI: lymphovascular invasion; LN: Lymph node; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor-2

The high NLR was significantly associated with high grade, LVI, LNs positivity, advanced stage, HER2 positivity, and high Ki-67 expression. The high PLR correlated with tumor grade, LVI, LNs status, AJCC stage, and Ki-67 expression significantly.

The median absolute neutrophil count was 3.4×10^3 cells/µL (range, 2.1×10^3 - 8.2×10^3 cells/µL) with the mean count was $3.8 \times 10^3 \pm 1.3 \times 10^3$ cells/µL. The median absolute platelet count was 300×10^3 cells/µL (range, 100×10^3 - 450×10^3 cells/µL) with the mean count was $298.2 \times 10^3 \pm 80.1 \times 10^3$ cells/µL. The median lymphocyte count was 1.6×10^3 (range, 0.66×10^3 - 4.7×10^3 cells/µL) with the mean count was $1.7 \times 10^3 \pm 0.7 \times 10^3$ cells/µL.

The median NLR was 2.17 (range, 1.14 - 7.0) and the mean value was 2.45±1.027. The median PLR was 171.4 (range, 55.2 - 621.2) and the mean value was 197.7±100.6. The sensitivity and specificity of NLR were 57.9% and 62.7%, respectively, and were 52.5% and 57.3% for PLR, respectively. The positive and negative predictive values (PPV and NPV) for NLR were 54.8% and 65.2%, and were 49.2% and 60.5% for PLR, respectively.

By using ROC curve analysis we determined cut-off values of NLR and PLR to predict DFS (Figures 1 & 2). The ROC curve analysis suggested that the cut-off value of 2.2 for NLR was the best to distinguish between patient's DFS (area under the curve AUC: 0.617, 95% CI: 0.521–0.714). A cut-off value of 180.0 for PLR was the best to discriminate between patient's DFS (AUC: 0.585, 95% CI: 0.488–

0.682). Sixty-four (47.8%) patients had NLR cut-off values ≥2.2 and 63 (47.0%) patients had PLR cut-off values ≥180.0.

The median number of NAC cycles was 4 (range 2–6). No clinical complete response (cCR) was detected. However, pathological CR including the LNs status was found in 7 patients (5.2%). The overall clinical response rate (CR+PR) was 47.8% that was significantly correlated with the lower value of both NLR and PLR (p<0.001 for both) (Table 2).

At the end of the study, 98 (73.1%) patients were alive with 70 months median follow-up for all patients (range 15–99 months). Seventy (52.2%) patients were free from recurrence while local relapse developed in 8 (6%) patients, distant relapse developed in 44 (32.8%) patients, and both local and distant relapse developed in 12 (9%) patients. Distant metastases in solitary and multiple sites were seen in 35 (26.1%) and 21 (15.7%) patients respectively. The initial sites of metastases were as follows: bone (23.1%), liver (15.7%), brain (10.4%), lung (7.5%), and others (4.5%). The loss to follow-up rate was 3.2% after 3 years and 7% after 5 years.

During follow up, the rate of relapse was 38.6% and 57.8% in low and high NLR patient groups, respectively while it was 43.7% and 52.4% in low and high PLR patient groups, respectively.

As regards the survival outcome, the median DFS was 72 months (95% CI, 58.1-85.9) for all patients with 69.7% 5-year DFS rate. For the NLR, the estimated 5-year DFS rates were 92.7% and 43.9%,

for low versus high ratio groups, respectively (*p*<0.001) (Figure 3). For the PLR, the estimated 5-year DFS rates were 82.8% and 55.2%, for low versus high ratio groups, respectively (*p*=0.008) (Figure 4).

On univariate analysis, both high values of NLR and PLR had a significant association with poor DFS. On multivariate analysis, this significance remains for NLR (HR: 3.2, 95% CI: 1.7-5.9, *p*<0.001), but not for

PLR (HR: 0.8, 95% CI: 0.5-1.6, *p*=0.595). In addition, multivariate analysis revealed that LNs involvement and high Ki-67 level had a significant association with lower DFS (Table 3). Low NLR had a significant association with better DFS for the luminal B, HER2, and TNBC subtypes (Table 4). However, there was no significant difference in the DFS according to PLR among the different intrinsic subtypes (Table 5).
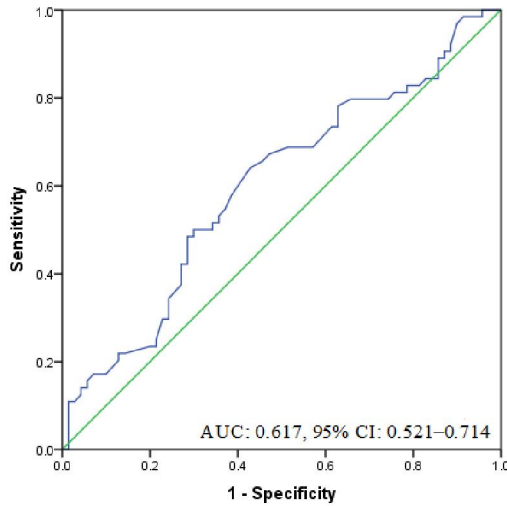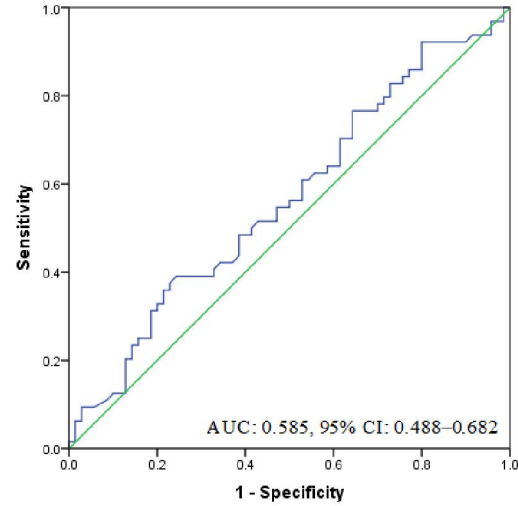

Figure 1: ROC curve analysis and AUC for NLR

Figure 2: ROC curve analysis and AUC for PLR.

Table 2. Relationship between baseline NLR & PLR and clinical response

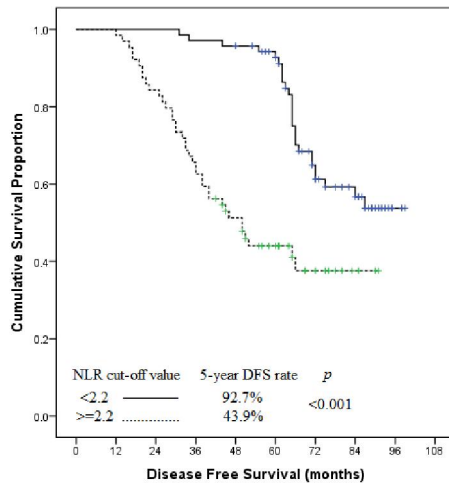| Baseline values	Clinical response				*p*
	CR (*n*=0) No. (%)	PR (*n*=64) No. (%)	SD (*n*=60) No. (%)	PD (*n*=10) No. (%)	
NLR <2.2 (*n*=70) ≥2.2 (*n*=64)	0 (0) 0 (0)	50 (71.4) 14 (21.9)	19 (27.1) 41 (64.1)	1 (1.4) 9 (14.1)	<0.001
PLR <180 (*n*=71) ≥180 (*n*=63)	0 (0) 0 (0)	46 (64.8) 18 (28.6)	23 (32.4) 37 (58.7)	2 (2.8) 8 (12.7)	<0.001

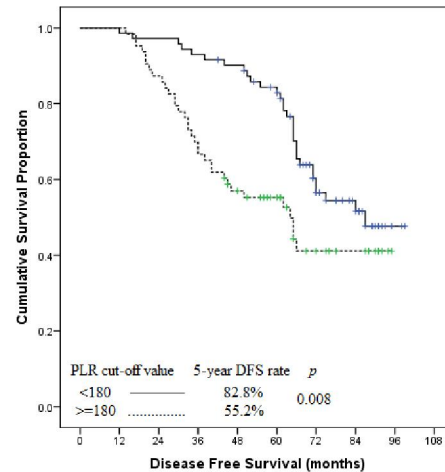**Figure 3.** DFS rate according to NLR.

Figure 4. DFS rate according to PLR

Table 3. Uni- and multivariate analysis of clinicopathological parameters affecting DFS

Parameters		5-year DFS rate	Median DFS (months)	Univariate analysis		Multivariate analysis	
				95% CI	*p*	HR (95% CI)	*p*
Age (years)	≤50	71.3%	84	65.3-102.7	0.613	---	---
	>50	69.0%	66	58.2-73.8			
Menopausal status	Menopause	68.6%	67	NR	0.717	---	---
	Premenopausal	71.1%	84	66.0-101.9			
Histology	IDC	68.8%	71	55.9-86.1	0.293	---	---
	ILC	85.7%	NR	NR			
Tumor grade	G1 & 2	82.4%	87	NR	**<0.001**	1.0 (0.7-1.4)	0.977
	G3	47.1%	45	19.0-71.0			
LVI	Yes	54.2%	65	50.8-79.2	**<0.001**	1.0 (0.6-2.0)	0.882
	No	87.2%	NR	NR			
Tumor size	≤2	89.9%	NR	NR	0.043	2.3 (0.7-7.3)	0.177
	>2	66.0%	66	58.9-73.1			
Lymph node status	N0	97.5%	NR	NR	**<0.001**	2.7 (1.1-6.6)	**0.025**
	N1 & 2	57.9%	65	61.3-68.7			
AJCC stage	IIA	94.5%	NR	NR	**<0.001**	1.7 (0.9-3.1)	0.069
	IIB	76.9%	72	63.6-80.4			
	IIIA	39.5%	35	16.1-54.0			
HR	+ve	73.7%	84	NR	0.006	1.4 (0.6-3.1)	0.424
	-ve	52.9%	62	23.6-100.4			
HER2 status	+ve	37.7%	51	34.8-67.2	**<0.001**	0.6 (0.3-1.4)	0.242
	-ve	76.2%	87	NR			
Ki-67 values	<14%	88.7%	NR	NR	**<0.001**	3.7 (1.4-9.8)	**0.009**
	≥14%	46.7%	50	24.1-75.9			
Subtype	Luminal A	91.5%	NR	NR	**<0.001**	0.9 (0.6-1.2)	0.432
	Luminal B	51.8%	61	47.7-74.3			
	HER2	68.8%	65	59.6-70.4			
	TNBC	33.3%	19	0.0-42.8			
NLR	<2.2	92.7%	NR	NR	**<0.001**	3.0 (1.7-5.5)	**<0.001**
	≥2.2	43.9%	50	37.9-62.1			
PLR	<180	82.8%	87	NR	0.008	0.9 (0.5-1.7)	0.771
	≥180	55.2%	64	45.8-82.2			

NR: not reported

Table 4: DFS rate of BC patients according to intrinsic subtypes in correlation with NLR

Pathological subtypes	NLR	5-year DFS rate	Median DFS (month)	95% CI	*p*
Luminal A	<2.2	97.7%	NR	NR	0.423
	≥2.2	81.1%	NR	NR	
Luminal B	<2.2	89.4%	NR	NR	<0.001
	≥2.2	25.0%	38	32.8-43.2	
HER2	<2.2	83.3%	66	NR	0.014
	≥2.2	28.6%	51	18.9-83.1	
TNB	<2.2	60.0%	66	0-137.7	0.029
	≥2.2	14.3%	17	15.8-18.2	

Table 5: DFS rate of BC patients according to intrinsic subtypes in correlation with PLR

Pathological subtypes	PLR	5-year DFS rate	Median DFS (month)	95% CI	p
Luminal A	<180	97.2%	NR	NR	0.215
	≥180	82.4%	NR	NR	
Luminal B	<180	73.7%	72	NR	0.057
	≥180	37.9%	40	33.0-47.0	
HER2	<180	76.2%	66	60.7-71.3	0.291
	≥180	60.0%	NR	NR	
TNB	<180	33.3%	31	9.4-52.6	0.559
	≥180	33.3%	17	13.0-21.0	

4. Discussion

Recent studies confirmed worse prognosis and progression of different types of cancers including BC in association with inflammatory responses. The presence of TILs is associated with a better response of the BC to cytotoxic agents with a better prognosis. Hematological parameters, such as NLR and PLR, were applied to evaluate the inflammatory response. However, the prognostic effects of NLR and PLR in early BC patients is still a matter of debate [13]. So, we aimed to assess the prognostic effect of NLR and PLR in an early resectable stage, non-metastatic BC patients treated with NAC.

In this study, the NLR was significantly correlated with the tumor grade, LVI, positive nodes, AJCC stage, positive HER2 expression, and Ki-67 expression (≥14%). Noh et al. [14] analyzed pretreatment NLR of over 400 BC patients in Korea and reported that high NLR was significantly associated with tumor grade 3, the presence of LVI, and demonstrated that patients with NLR ≥ 2.5 was associated with younger age, increased T stage, and positive HER2 expression. Dirican et al. [15] reported that tumor depth (pT), positive nodes, increasing AJCC pathological stage and presence of distant metastases associated with high NLR significantly. However, Yersal et al. [16] and Ulas et al. [1] did not identify any significant correlations among clinical and pathological parameters and the NLR in patients with BC.

As regards the significant correlations between the PLR and the clinicopathological characteristics we found that, elevated PLR was significantly correlated with tumor grade 3, the presence of LVI, positive nodes, disease stage IIIA and high Ki-67 value (≥14%). Yersal et al. [16] reported that higher PLR correlated with age and tumor diameter. Asano et al. [17] analyzed 177 early BC patients treated with NAC. The low-PLR group had significantly more patients >56 years old and postmenopausal women than the high-PLR group and associated with a higher pCR rate.

In our study, we recorded 5.2% of all patient had pCR and as the result of their small number, we don't correlate the pCR rate with NLR and PLR. On the other hand, the overall clinical response rate was 47.8% that significantly correlated with both NLR and PLR. Suppan et al. [18] treated selected early-stage BC patients with preoperative systemic treatment and reported that elevated NLR does not predict response nor correlate with the prognosis. Asano et al. [17] found that the low PLR group had a significantly higher pCR rate.

Several studies used the means, medians, and quartiles that allow dividing the studied patients into groups. The ROC curve analysis is a design allowing probing individual values according to the end result (in this study, the treatment failure). By using ROC curve analysis, the cut-off value determination provides the best prediction of the sensitivity and specificity. Consequently, ROC curve analysis that applied in the present study is the most appropriate approach.

There is no general agreement for threshold or cut-off value for the NLR or PLR to be applied for the risk of recurrence. However, in the present study, we suggested that, 180 was an optimal PLR cut-off value to discriminate between DFS. Few studies had concerned with PLR as a prognostic indicator in BC. However, Krenn-Pilko et al. [19] determined 292 as the optimal PLR cut-off value to discriminate between cancer-specific survival (CSS), overall survival (OS), and distant metastases-free survival (DMFS) and it was 147 in Liu et al. [20] study for discrimination between OS, and DFS.

The NLR (2.2) among our patients was lower than that of most of the published studies such as Dirican et al. [15] (NLR; 4) Asano et al. [21] (NLR; 3) and Pistelli et al et al. [22] (NLR; 3). It was higher than that reported in the studies of Zhang et al. [23] (NLR; 1.81), Hong et al. [24] (NLR; 1.93) and Jia et

al. [25] (NLR; 2). An explanation for these different results may contribute to differences in race and study population, for example; our patients had a limited stage of disease, at the same time included the all molecular subtypes.

In the current trial, we assessed the prognostic value of the inflammatory response parameters as regard DFS and not OS which can be influenced by numerous other factors including non-cancer-related death. The univariate analysis showed a significant association of high NLR and PLR with poor DFS. On multivariate analysis only the significant prognostic value of high NLR continued. Noh et al. [14] reported that elevated NLR was associated with poor disease-specific survival. Yao et al. [26] showed that elevated NLR (≥2.5) significantly associated with poor prognosis. Azab et al. [27] used NLR and PLR quartiles for stratification of survival in BC patients. Patients with the highest NLR and PLR quartiles (4th quartiles) had a higher 5-year mortality rate compared to the patients in the other three quartiles with lower NLR and PLR. Pretreatment NLR was considered as an independent predictor factor of long-term mortality. Dirican et al. [15] retrospectively analyzed the predictive or prognostic role of the preoperative NLR in 1,527 BC patients and reported the independent prognostic effect on OS and DFS. Krenn-Pilko et al. [19] showed that a high PLR is a consistent factor for poor prognosis, while an elevated NLR significantly associated with CSS in univariate analysis but not in multivariable analysis. Koh et al. [28] reported that a high pretreatment NLR significantly associated with overall death among BC patients. Finally, Chen et al. [29] conducted a meta-analysis of five studies and demonstrated that a high pretreatment NLR, with the cut-off values, ranged between 2.0 and 4.0, were correlated with a significant poor OS, but to a lesser extent with DFS.

In the current trial, there was no significant difference of the DFS for the different BC intrinsic subtypes according to PLR. On the other hand, there was a significant difference of the DFS for the luminal B, HER2 and TNBC subtypes according to NLR. Several studies reported different outcomes where Koh et al. [28] assessed the prognostic value of NLR in 157 HER2-negative and HR-positive BC patients treated with preoperative CT. They found that NLR >2.25 was correlated with poor OS and recurrence-free survival. Krenn-Pilko et al. [19] reported a significant association of the elevated PLR with CSS in luminal B tumors. Liu et al. [20] studied 318 HR-negative, non-metastatic BC patients and reported that NLR significantly correlated with OS and DFS, but PLR was not.

The TNBCs have genomic instability with more mutations and heterogeneous immune cells infiltration.

Systemic inflammatory response parameters are considered as predictive markers of outcomes for TNBC patients in several studies. Subgroup analysis in Liu et al. [20] study revealed a significant association between increased NLR and PLR and poor survival in TNBC. Pistelli et al. [22] evaluated the prognostic effect of pretreatment NLR in early TNBC patients. Patients with high NLR (>3) had a significantly worse DFS and OS than patients with low NLR (≤3). Jia et al. [25] and Asano et al. [21] found that high pretreatment NLR was significantly correlated with a poor prognosis in patients with TNBC.

Multivariate analysis of our results revealed that LNs involvement and high Ki-67 were significantly associated with poor DFS. Orditura et al. [30] found that premenopausal status and the N1 stage had independent prognostic factors with poor recurrence rate. Liu et al. [20] recorded that, tumor size ≥2 cm and grade 3 tumors significantly associated with poor DFS.

The limitations of this study included; a small number of patients in a single center with a relatively short follow-up period (median follow-up; 98 months). Also, the retrospective analysis of our study made us unable to analyze some clinicopathological parameters that may be related to NLR and PLR such as intense physical exercise, severe stress, and malnutrition. Although patients with disorders that may influence NLR and PLR were excluded, there might be some studied patients suffer from other disorders not included in the patients' medical files.

Conclusion:

The high NLR significantly correlated with poor DFS in patients with early non-metastatic BC treated with NAC, but PLR is not. As NLR is a clinical marker that can be easily applied and its high value was correlated with poor prognosis of early BC patients, NLR might be a potential predictor in patients' outcome to assist in treatment decisions.

5. Conflict of Interest
The authors declare no conflict of interest

References
1. Ulas A, Avci N, Kos T, Cubukcu E, Olmez OF, Bulut N, et al. Are neutrophil/lymphocyte ratio and platelet/lymphocyte ratio associated with prognosis in patients with HER2-positive early breast cancer receiving adjuvant trastuzumab? J BUON. 2015; 20(3):714-22.
2. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860-7.

3. Proctor MJ, McMillan DC, Morrison DS, Fletcher CD, Horgan PG, Clarke SJ. A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer. Br J Cancer. 2012;107(4):695-9.

4. Guthrie GJ, Charles KA, Roxburgh CS, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil lymphocyte ratio: experience in patients with cancer. Crit Rev Oncol Hematol. 2013; 88(1):218-30.

5. Yamanaka T, Matsumoto S, Teramukai S, Ishiwata R, Nagai Y, Fukushima M. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in advanced gastric cancer. Oncology. 2007; 73(3-4):215-20.

6. De Larco JE, Wuertz BR, Furcht LT. The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. Clin Cancer Res. 2004;10(15):4895-900.

7. Sylman JL, Mitrugno A, Atallah M, Tormoen GW, Shatzel JJ, Tassi Yunga S, et al. The Predictive Value of Inflammation-Related Peripheral Blood Measurements in Cancer Staging and Prognosis. Front Oncol. 2018;8:78.

8. Nieswandt B, Hafner M, Echtenacher B, Männel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. Cancer Res. 1999; 59(6):1295-300.

9. Klinger MH, Jelkmann W. Role of blood platelets in infection and inflammation. J Interferon Cytokine Res. 2002; 22(9):913-22.

10. Salazar-Onfray F, Lopez MN, Mendoza-Naranjo A. Paradoxical effects of cytokines in tumor immune surveillance and tumor escape. Cytokine Growth Factor Rev. 2007; 18(1-2):171-82.

11. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348(3):203-13.

12. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013; 24(9):2206-23.

13. Faria SS, Fernandes PC Jr, Silva MJ, Lima VC, Fontes W, Freitas-Junior R, et al. The neutrophil-to-lymphocyte ratio: a narrative review. E cancer medical science. 2016;10:702.

14. Noh H, Eomm M, Han A. Usefulness of pretreatment neutrophil to lymphocyte ratio in predicting disease-specific survival in breast cancer patients. J Breast Cancer. 2013;16(1):55–9.

15. Dirican A, Kucukzeybek BB, Alacacioglu A, Kucukzeybek Y, Erten C, Varol U, et al. Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer?. Int J Clin Oncol. 2015;20(1):70-81.

16. Yersal Ö, Çetinkünar S, Aktimur R, Aziret M, Özdaş S, Erdem H, et al. Neutrophil/Lymphocyte and Platelet/Lymphocyte Ratios are Not Different among Breast Cancer Subtypes. Asian Pac J Cancer Prev. 2017;18(8):2227-31.

17. Asano Y, Kashiwagi S, Onoda N, Noda S, Kawajiri H, Takashima T, et al. Platelet-Lymphocyte Ratio as a Useful Predictor of the Therapeutic Effect of Neoadjuvant Chemotherapy in Breast Cancer. PLoS One. 2016;11(7):e0153459.

18. Suppan C, Bjelic-Radisic V, La Garde M, Groselj-Strele A, Eberhard K, Samonigg H, et al. Neutrophil/Lymphocyte ratio has no predictive or prognostic value in breast cancer patients undergoing preoperative systemic therapy. BMC Cancer. 2015;15:1027.

19. Krenn-Pilko S, Langsenlehner U, Thurner EM, Stojakovic T, Pichler M, Gerger A, et al. The elevated preoperative platelet-to-lymphocyte ratio predicts poor prognosis in breast cancer patients. Br J Cancer. 2014; 110(10):2524–30.

20. Liu C, Huang Z, Wang Q, Sun B, Ding L, Meng X, et al. Usefulness of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in hormone-receptor-negative breast cancer. Onco Targets Ther. 2016; 9:4653-60.

21. Asano Y, Kashiwagi S, Onoda N, Noda S, Kawajiri H, Takashima T, et al. Predictive Value of Neutrophil/Lymphocyte Ratio for Efficacy of Preoperative Chemotherapy in Triple-Negative Breast Cancer. Ann Surg Oncol. 2016;23(4):1104-10.

22. Pistelli M, De Lisa M, Ballatore Z, Caramanti M, Pagliacci A, Battelli N, et al. Pre-treatment neutrophil to lymphocyte ratio may be a useful tool in predicting survival in early triple negative breast cancer patients. BMC Cancer. 2015;15:195.

23. Zhang P, Zong Y, Liu M, Tai Y, Cao Y, Hu C. Prediction of outcome in breast cancer patients using test parameters from complete blood count. Mol Clin Oncol. 2016; 4(6):918–24.

24. Hong J, Mao Y, Chen X, Zhu L, He J, Chen W, et al. Elevated preoperative neutrophil -to-lymphocyte ratio predicts poor disease-free

survival in Chinese women with breast cancer. Tumour Biol. 2016;37(3):4135–42.

25. Jia W, Wu J, Jia H, Yang Y, Zhang X, Chen K, et al. The Peripheral Blood Neutrophil-To-Lymphocyte Ratio Is Superior to the Lymphocyte-To-Monocyte Ratio for Predicting the Long-Term Survival of Triple-Negative Breast Cancer Patients. PLoS One. 2015;10(11):e0143061.

26. Yao M, Liu Y, Jin H, Liu X, Lv K, Wei H, et al. Prognostic value of preoperative inflammatory markers in Chinese patients with cancer. Onco Targets Ther. 2014; 7:1743-52.

27. Azab B, Shah N, Radbel J, Tan P, Bhatt V, Vonfrolio S, et al. Pretreatment Neutrophil/Lymphocyte Ratio Is Superior to Platelet/Lymphocyte Ratio as a Predictor of

Long-Term Mortality in Breast Cancer Patients. Med Oncol. 2013; 30(1):432.

28. Koh YW, Lee HJ, Ahn JH, Lee JW, Gong G. Prognostic significance of the ratio of absolute neutrophil to lymphocyte counts for breast cancer patients with ER/PR-positivity and HER2-negativity in neoadjuvant setting. Tumour Biol. 2014; 35(10):9823-30.

29. Chen J, Deng Q, Pan Y, He B, Ying H, Sun H, et al. Prognostic value of neutrophil-to-lymphocyte ratio in breast cancer. FEBS Open Bio. 2015; 5:502-7.

30. Orditura M, Galizia G, Diana A, Saccone C, Cobellis L, Ventriglia J, et al. Neutrophil to lymphocyte ratio (NLR) for prediction of distant metastasis-free survival (DMFS) in early breast cancer: a propensity score-matched analysis. ESMO Open. 2016;1(2):e000038.

2/4/2019