RESEARCH ARTICLE

Neutrophil/Lymphocyte and Platelet/Lymphocyte Ratios are Not Different among Breast Cancer Subtypes

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Abstract

Background: Breast cancer is a heterogeneous complex of diseases comprising different subtypes that have different treatment responses and clinical outcomes. Systemic inflammation is known to be associated with poor prognosis in many types of cancer. The neutrophil / lymphocyte ratio (NLR) and platelet / lymphocyte ratio (PLR) are factors used as indicators of inflammation. In this study, we evaluated NLR and PLR ratios in breast cancer subtypes. **Methods:** A total of 255 breast cancer patients were evaluated retrospectively. Patients were classified into three subtypes: estrogen receptor (ER)- or progesterone receptor (PR)-positive tumors were classified as luminal tumors; human epidermal growth factor receptor-2 (HER2)-overexpressed and ER-negative tumors were classified as HER2-positive tumors; and ER, PR, and HER2-negative tumors were classified as triple-negative tumors. The NLR and PLR were calculated. **Results:** The median NLR and PLR were 3 (0.37–37,1) and 137 (37.1–421.3), respectively. 66.7% of the patients were luminal type, 19.2% were HER2 positive, and 14.1% were triple negative. NLR was not associated with grade (p: 0.412), lymphovascular invasion (p: 0.326), tumor size (p: 0.232) and metastatic lymph node involvement (p: 0.406). PLR was higher in the patients with lymph node metastasis than in those without lymph node metastasis (p: 0.03). The NLR was 2 in the luminal group, 1.8 in the HER2-positive group, and 1.9 in the triple-negative group, but the differences were not significant(p: 0.051). PLR was 141 in the luminal group, 136 in the HER2-positive group, and 130 in the triple-negative group, but the differences were not significant. **Conclusion:** We could not find any significant differences for NLR and PLR according to breast cancer subtypes.

Keywords: Breast cancer- CerbB2- Estrogen receptor- Progesterone receptor- Neutrophil/lymphocyte ratio (NLR)

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Introduction

Breast cancer is a heterogeneous complex of diseases consisting of many subtypes with distinct biological features. Breast cancer subtypes have different response patterns to various treatment modalities and clinical outcomes. Traditional classification systems regarding biological characteristics may have limitations for patient-tailored treatment strategies. Breast cancers have been classified into different intrinsic subtypes with distinct clinical outcomes according to gene expression patterns, and each intrinsic subtype is represented by an immunohistochemical-defined subtype (Yersal et al., 2014).

Tumor characteristics and host response, including the inflammatory response, both contribute to the clinical outcomes of cancer patients. Histopathological analysis of tumors has shown tumor infiltration by inflammatory and lymphocytic cells. Immune infiltrates are heterogeneous between tumor types and differ from patient to patient. The interaction of the immune system with tumor cells in breast cancer appears to be associated with prognosis (Faria et al., 2016). The neutrophil / lymphocyte ratio (NLR) and platelet / lymphocyte ratio (PLR) are easily measured, reproducible, and inexpensive markers of subclinical inflammation and have a prognostic role in various cancer types, including breast cancer.

Human epidermal growth factor receptor-2 (HER2) positivity has been shown to be associated with higher NLR, whereas estrogen receptor (ER)- and progesterone receptor (PR)-positivity have been associated with lower NLR values (Dirican et al., 2015). HER2-positive and triple-negative breast cancer (TNBC) are thought to be more immunogenic than luminal carcinomas (Luen and Loi, 2016). The aim of this study was to investigate the NLR and PLR values in different molecular subtypes of breast cancer.

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Materials and Methods

In this study, we retrospectively evaluated a total of 255 female breast cancer patients who underwent surgery at the Adana Numune Training and Research Hospital and Samsun Training and Research Hospital from January 2010 to December 2014. The study began following approval by the Academic Committee. Preoperative complete blood counts (leukocytes, neutrophils, lymphocytes) of the patients with histologically verified breast cancer were analyzed. Patients with active infection, active bleeding, blood transfusion within the last 3 months, chronic inflammatory or autoimmune disease, or steroid treatment were excluded from the study.

Medical records were collected from medical files. Medical history, age, and subtype of breast cancer, the number of axillary lymph node metastases, tumor diameter, TNM stage, status and positivity of ER and PR, HER2, complete blood cell counts, and NLR and PLR were obtained for each patient. ER and PR status were attained from immunohistochemistry, and a value ≥1% was considered as positive. HER2 status was obtained by immunohistochemistry or fluorescent in situ hybridization (FISH). IHC 3+ and IHC 2+ that were FISH positive were classified as HER2-positive tumors; IHC 2+ that were either FISH negative, IHC 1+, or IHC positive were classified as HER2-negative tumors.

Intrinsic breast cancer subtypes were classified according to the following criteria: luminal subtype, ER positive, and/or PR positive and HER2 negative, HER2-enriched subtype, ER, and PR negative with positive HER2, triple-negative tumors, ER negative, PR negative, and HER2 negative.

Complete blood count test results were attained within 1 week before the surgery. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. PLR was defined as the absolute platelet count divided by the absolute lymphocyte count. The patients were divided into two groups according to the mean value of NLR (low NLR: ≤2.5, high: NLR: >2.5).

Statistical analysis

The data analysis was performed by using SPSS for Windows, version 22 (SPSS, Chicago, IL, USA). The normality of the distributions of continuous variables was determined via the Shapiro–Wilk test. The data were reported as the mean ± standard deviation or median and range where applicable. The differences in the results between the groups were compared by performing Student's t-test or the Mann–Whitney U test, where appropriate. When more than two independent groups were considered, the Kruskal–Wallis test was applied for comparisons of the median values. The categorical data were analyzed by using Pearson's chi-square or Fisher's exact test, where appropriate. Degrees of association between continuous variables were evaluated via Spearman's correlation analysis. The coefficient of regression and the 95% confidence interval for each independent variable were also calculated. A *P value* < 0.05 was considered as indicating statistical significance.

Table 1. Patients Characteristics

Characteristics	Median	Min-max
Age (years)	53	24– 76
Tumor diameter	3	0.1– 11
Metastatic lymph node	1	0– 37
Total lymph node	16	1– 37
NLR	2.01	0.37– 37.1
PLR	137.8	37.1– 421.3

Results

Between January 2010 and December 2014, 255 breast cancer patients were enrolled in the study. All patients were female, and the median age was 53. The baseline characteristics of the study subjects are summarized in Table 1. The most common histological type was invasive ductal carcinoma. Stage II was the most common stage. The median values of NLR and PLR were 2.01 (range, 0.37–37.1) and 137.8 (range, 37.1–421.3), respectively. Of the total 255 patients, 183 had NLR < 2.5 and 72 patients had NLR ≥ 2.5.

Breast cancer histopathological features and subtypes are shown in Table 2. The most defined molecular subtype was luminal type. The HER2-positive group accounted for 19.2% of all patients, and the triple-negative group accounted for 14.1%.

PLR was positively correlated with tumor size and

Table 2. Breast Cancer Histopathological Features and Subtypes

Features		N (%)
Histology (n = 255)		
	Invasive ductal	202 (79.2)
	Invasive lobular	18 (7.1)
	Others	35 (13.7)
Stage (n = 255)		
	1A	29 (11.4)
	1B	4 (1.6)
	2A	60 (23.5)
	2B	52 (20.4)
	3A	50 (19.6)
	3B	25 (9.8)
	3C	28 (11)
	4	7 (2.7)
ER	Positive	195 (76.5)
	Negative	60 (23.5)
PR	Positive	170 (66.7)
	Negative	85 (33.3)
Cerb-B2	Positive	49 (20.4)
	Negative	206 (79.6)
Subtype	Luminal	170 (66.7)
	HER2 positive	49 (19.2)
	Triple negative	36 (14.1)

Table 3. Tumor Characteristics and NLR/PLR Correlation

Variables	NLR	PLR
Age (years)	r = −0.021 P = 0.734	r = −0.166 P = 0.008
Tumor diameter	r = −0.091 P = 0.149	r = 0.138 P = 0.028
Metastatic lymph node	r = 0.068 P = 0.276	r = 0.075 P = 0.233
Total lymph node	r = −0.060 P = 0.338	r = −0.061 P = 0.334
Ki-67	r = −0.015 P = 0.875	r = 0.005 P = 0.961

Table 4. Tumor and Patient Characteristics by NLR

Characteristics		NLR		*P*
	Patients	<2.5 (n = 183)	≥2.5 (n = 72)	
Age (years)	255	55 (27–75)	53 (26–75)	0.69
Tumor size	255	3 (0.1–11)	3 (0.2–11)	0.232
Metastatic lymph node	255	1 (0–37)	2 (0–23)	0.406
Total lymph node	255	17 (1–37)	14 (3–34)	0.123
Histological grade	212	150	62	
Grade 1	22	18 (12.0)	4 (6.5)	0.412
Grade 2	99	67 (44.7)	32 (51.6)	
Grade 3	91	65 (43.3)	26 (41.9)	
Lenfovascular invasion	186	130	56	
No	46	29 (22.3)	17 (30.4)	0.326
Yes	140	101 (77.7)	39 (69.6)	
Perineural invasion	139	99	40	
No	75	56 (56.6)	19 (47.5)	0.434
Yes	64	43 (43.4)	21 (52.5)	
ER	255			
Negative	60	47 (25.7)	13 (18.1)	0.259
Positive	195	136 (74.3)	59 (81.9)	
PR	255			
Negative	85	65 (35.5)	20 (27.8)	0.302
Positive	170	118 (64.5)	52 (72.2)	
Cerb B2	252	181	71	
Negative	203	141 (77.9)	62 (87.3)	0.128
Positive	49	40 (22.1)	9 (12.7)	

age. There were no significant correlations among NLR or PLR and metastatic lymph node count, total lymph node count, or Ki-67 status (Table 3).

The associations among NLR and clinicopathological variables are shown in Table 4. NLR had no statistically significant associations with age, HER2 expression status, or hormone receptor positivity. There was no differences in tumor size; ER, PR, and cerbB2 status; histological grade; and lymphovascular and perineural invasion between the low- and high-NLR groups.

There were no significant differences in the NLR and PLR values among the luminal type, HER2-positive, or triple-negative tumors (Table 5).

Table 5. Breast Cancer Subtypes According to Low- and High-NLR Values

Subtype	NLR		
	<2.5	>2.5	*P*
Luminal	113 (66.9)	56 (33.1)	0.051
HER2 positive	41 (82)	9 (18)	
Triple negative	29 (80.6)	7 (19.4)	

Discussion

In this study, we examined the relationships among breast cancer subtypes and the NLR and PLR as indicators of inflammation. Several studies have shown that, compared with the luminal A subtype, the triple-negative and HER2-enriched breast cancer subtypes are more immunogenic and associated with poorer prognosis (Liu,2014). A neutrophilic host response to tumor has also been associated with poor prognosis because it can inhibit the immune system by suppressing the cytotoxic activity of T cells. We hypothesized that the degree of inflammatory response, as indicated by NLR, may be different among breast cancer subtypes. We could not find any differences in NLR and PLR values among breast cancer subtypes, including triple-negative and HER2-positive subtypes.

Several studies have demonstrated relationships between the inflammatory system and cancer. Pretreatment neutrophil, lymphocyte, and platelet counts are indicators of cancer-associated inflammation. High neutrophil count has 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 (Jia et al., 2015; Li et al., 2015; Stotz et al., 2013; Ishizuka et al., 2013; Peng et al., 2015; Kim et al., 2015). The interaction between neutrophil and lymphocyte inflammatory responses has a critical role in carcinogenesis. The mechanism underlying the relationship between a high NLR and poor outcome in breast cancer is not completely understood. A multifactorial process has been hypothesized to explain the relationship between high NLR and poor prognosis. Neutrophils may act as tumor-promoting leukocytes and be capable of inhibiting the immune system via suppression of the cytolytic activity of lymphocytes, natural killer cells, and activated T-cells. Neutrophils promote leakage of tumor cells and endothelial cells into the circulation, thereby contributing to redirection of the inflammatory response in a tumor-promoting direction (Galdiero et al., 2013). Neutrophils can also secrete circulating vascular endothelial growth factor that promotes tumor development (Balkwill et al., 2012); therefore, an elevated neutrophil count can stimulate tumor angiogenesis and contribute to disease progression, thus leading to a negative correlation between neutrophil density and patient survival. Neutrophilia can also be due to paraneoplastic production of myeloid growth factors produced by cancer (Teramukai et al., 2009). These factors inhibit apoptosis of tumor cells via nuclear factor kB activation. These events enhance angiogenesis, tumor

growth, and progression to a metastatic phenotype (Quail and Joyce, 2013). Thus, the NLR can reflect the balance between activation of the inflammatory pathway and the anti-tumor immune function in breast cancer.

Absolute neutrophil and lymphocyte numbers may be influenced by various physiological, pathological, and physical factors. However, NLR has been shown to remain more stable than other leukocyte parameters. NLR and PLR are easily available simple markers based on white blood cell count and platelet numbers that indicate subclinical inflammation. Noh et al., (2013) found that elevated NLR at initial clinical presentation of breast cancer was an independent factor for poor survival rate in breast cancer patients. In addition, Yao et al. showed that elevated NLR (≥2.5) and RDW were significantly associated with poor prognosis (Yao et al., 2014). In this study, we performed a cohort of breast cancer patients to evaluate the NLR and PLR values in different molecular subtypes of breast cancer.

Several studies have reported different outcomes according to NLR values between breast cancer subtypes. Koh et al., (2014) evaluated 157 hormone receptor-positive HER2-negative breast cancer patients treated with neoadjuvant chemotherapy, with a mean follow-up of 21 months after surgery. They showed that a NLR > 2.25 was associated with shorter recurrence-free and overall survival (OS) and was an independent risk factor for recurrence and mortality.

TNBCs show genetic instability leading to an increase in the number of mutations. Recent studies have shown that systemic inflammatory parameters could be helpful for predicting outcomes of TNBC patients. Pistelli et al., (2015) analyzed 90 patients with early TNBC to evaluate the correlations of NLR before treatment with disease-free survival (DFS) and OS. They showed that patients with a NLR > 3 had shorter DFS and OS than those of patients with a NLR ≤ 3. Jia et al., (2015) reported that a high NLR before treatment was independently associated with a worse prognosis for TNBC. TNBCs have genomic instability, so they have a tendency to generate neoantigens and result in a heterogeneous pattern of immune infiltration.

The clinicopathological features associated with high NLR values were increased T stage, younger age, and positive HER2 status. Azab et al., (2013) evaluated the prognostic ability of the NLR in 400 BC patients and showed that patients with a higher NLR were older and had more lymph node involvement and metastases Dirican et al., (2015) showed that tumor depth (pT), nodal status, American Joint Committee on Cancer staging (increasing pathological stage), and distant metastasis status were found to be significantly associated with high NLR. We found that higher PLR values were associated with age and tumor diameter, but we did not find any correlations among NLR and clinicopathological features. Ulas et al., (2015) did not identify any significant correlations among clinical and pathological parameters and the NLR in patients with BC.

Our study had some limitations that should be noted. A portion of the data was missing because of the retrospective nature of the study. The Ki 67 value was not evaluable in all patients, so those with luminal subtypes were not divided into Luminal A and Luminal B subgroups.

In conclusion, pretreatment NLR and PLR values were not associated with tumor subtype in breast cancer patients. Prospective studies with larger patient numbers and prognostic data will make it possible to obtain more reliable results.

Conflicts of Interest

None of the authors has a conflict of interest related to this study.

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