No impact of neutrophil-to-lymphocyte ratio on pathological response to neoadjuvant chemotherapy in Turkish women with breast cancer

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Abstract

A low neutrophil-to-lymphocyte ratio (NLR) reflects a systemic milieu characterized by reduced inflammation and increase immune system activation – potentially favoring a better response to therapy in solid malignancies. This retrospective study of collected data aimed to assess whether NLR may predict pathological complete response (pCR) to neoadjuvant chemotherapy (NAC) in Turkish women with breast cancer. The study sample consisted of 406 patients with breast cancer who were scheduled to undergo NAC before attempting cytoreductive surgery. A pretreatment complete blood count was obtained in the week preceding NAC, and the NLR was calculated from absolute counts of neutrophils and lymphocytes. The Miller-Payne system was used to grade response to NAC. Secondary outcome measures included disease-free survival (DFS) and overall survival (OS). Patients were divided into two groups (high versus low) according to the optimal cutoff value (NLR = 1.95; low NLR ≤ 1.95, high NLR > 1.95). The rate of pCR in the low NLR group (22.3%) was similar to that observed in the high NLR group (19.8%; P = 0.20). Moreover, there were no significant differences in DFS and OS of patients in the high and low NLR groups. Based on our results, pretreatment NLR seems to have no significant predictive value in Turkish women with breast cancer who received NAC. However, prospective studies will surely provide deeper insights into the prognostic significance of NLR in this clinical setting.

Introduction

Malignancies originating in the mammary gland are the most common type of cancer in women[1]. The lifetime risk of breast cancer for a woman has been calculated at around 1-in-7 to 1-in-10[2], indicating that approximately 10% of the female population will be diagnosed with breast cancer during their lifetime[1,2].

Neoadjuvant chemotherapy (NAC) – consisting of chemotherapy delivered before local treatment (surgery) – has become the standard of care in patients with locally advanced breast cancer[3]. In addition, downstaging of the primary tumor in patients with earlier stages of breast cancer may facilitate breast-conserving therapy and offer the opportunity to downstage the axilla – ultimately obviating the need for axillary treatment in some patients[4]. The overarching goal of NAC is to achieve pathological complete response (pCR) – which is, in turn, associated with a lower recurrence rate and more favorable survival outcomes[5]. Unfortunately, there is considerable interindividual variation in response to NAC amongst women with breast cancer, and several variables have been investigated in relation to this variability[6].

Inflammation has been shown to promote tumor initiation and progression, whereas escape from immune surveillance may favor cancer invasiveness[7]. There is also evidence that high tumor infiltration by neutrophils has an adverse prognostic significance, whereas tumor-infiltrating lymphocytes portend more favorable outcomes[8], especially in triple-negative and epidermal growth factor receptor (HER) 2-positive breast cancer[9]. The neutrophil-to-lymphocyte ratio (NLR) – has been extensively investigated in relation to prognosis in patients with different solid malignancies, including breast cancer[9]. In general, a low NLR reflects a systemic milieu characterized by reduced inflammation and increase immune system

activation – potentially favoring a better response to therapy[8,9]. Recently, Graziano et al.[10] have shown that the combined presence of a low NLR and a low platelet-to-lymphocyte ratio is predictive of pCR in Italian women with breast cancer treated with NAC. However, the question as to whether NLR may predict pCR to NAC as well as survival endpoints in Turkish women remains open. We, therefore, designed the current study to address these issues specifically.

Results

The general characteristics of the entire study sample (n = 406) before and after categorization according to low (n = 184) or high (n = 222) NLR are shown in Table 1. The two NLR groups did not differ significantly in terms of age and histopathology. There was a trend toward more frequent postmenopausal patients (P = 0.06) in the low NLR group (53.3% versus 48.3% in the high NLR group). With respect to treatment modalities, there were no intergroup differences with regard to the use of chemotherapy, radiotherapy, type of surgery, hormone therapy, and trastuzumab. Table 2 and Table 3 depict the pre- and post-treatment variables in different NLR groups. None of these variables showed significant intergroup differences.

Table 1
General characteristics of the study patients in different neutrophil-to-lymphocyte ratio groups (low *versus* high)

Characteristic	Entire cohort (n = 406)	Neutrophil-to-lymphocyte ratio		P
		Low (n = 184)	High (n = 222)	
Median age, years (minimum-maximum)	48.2 (22.0-83.5)	49.7 (23.9–77.4)	46.5 (22.0-83.5)	0.07
Menopausal status, n (%)	210 (51.7)	86 (46.7)	124 (51.7)	0.06
Premenopausal	196 (48.3)	98 (53.3)	98 (48.3)	
Postmenopausal				
Surgery, n (%)	305 (75.1)	144 (78.3)	161 (75.1)	0.18
Breast conservative surgery	101 (24.9)	40 (21.7)	61 (24.9)	
Mastectomy				
Histopathology, n (%)	369 (90.9)	167 (90.8)	202 (91.0)	0.93
Invasive ductal carcinoma	37 (9.1)	17 (9.2)	20 (9.0)	
*Other histology types**				
Chemotherapy, n (%)	97 (23.9)	42 (22.8)	55 (24.8)	0.53
Anthracycline regimens	20 (4.9)	7 (3.8)	13 (5.9)	
Taxane regimes	289 (71.2)	135 (73.4)	154 (69.3)	
Anthracycline plus taxane				
Hormone therapy, n (%)	97 (24.0)	45 (24.5)	52 (23.5)	0.83
No	308 (76.0)	139 (75.5)	169 (76.5)	
Yes				
Radiotherapy, n (%)	7 (1.7)	2 (1.1)	5 (2.3)	0.46
No	399 (98.3)	182 (98.9)	217 (97.7)	
Yes				

*Including lobular, mucinous, papillary, metaplastic, and mixed tumors. Data are given as counts (percentages) unless otherwise indicated.

Characteristic	Entire cohort (n = 406)	Neutrophil-to-lymphocyte ratio		P
		Low (n = 184)	High (n = 222)	
Trastuzumab, n (%)	296 (73.1)	129 (70.1)	167 (75.6)	0.19
No	109 (26.9)	55 (29.9)	54 (8.6)	
Yes				

*Including lobular, mucinous, papillary, metaplastic, and mixed tumors. Data are given as counts (percentages) unless otherwise indicated.

Table 2
Pre-treatment variables in different neutrophil-to-lymphocyte ratio groups (low *versus* high)

Characteristic	Entire cohort (n = 406)	Neutrophil-to-lymphocyte ratio		P
		Low (n = 184)	High (n = 222)	
Clinical T stage, n (%)	81 (20.5)	36 (20.5)	45 (20.5)	0.39
T1	202 (51.1)	96 (54.5)	106 (48.4)	
T2	26 (6.6)	8 (4.5)	18 (8.2)	
T3	86 (21.8)	36 (20.5)	50 (22.8)	
T4				
Clinical Node status, n (%)	161 (40.4)	76 (42.0)	85 (39.0)	0.54
Negative	238 (59.6)	105 (58.0)	133 (61.0)	
Positive				
ER expression, n (%)	101 (24.9)	49 (27.7)	52 (24.5)	0.48
Negative	288 (70.9)	128 (72.3)	160 (75.5)	
Positive	17 (4.2)			
Unknown				
PR expression, n (%)	152 (37.4)	70 (39.5)	82 (38.7)	0.86
Negative	237 (58.4)	107 (60.5)	130 (61.3)	
Positive	17 (4.2)			
Unknown				
HER-2 expression, n (%)0.52				
Negative	291 (71.7)	130 (73.4)	161 (76.3)	
Positive	97 (23.9)	47 (26.6)	50 (23.7)	
Unknown	18 (4.4)			
Ki-67 expression, n (%)	95 (23.4)	42 (25.6)	53 (27.3)	0.72
≤ 15%	263 (64.8)	122 (74.4)	141 (72.7)	
> 15%	48 (11.2)			
Unknown				
Data are given as counts (percentages).				

Table 3
Post-treatment variables in different neutrophil-to-lymphocyte ratio groups (low *versus* high)

Characteristic	Entire cohort (n = 406)	Neutrophil-to-lymphocyte ratio		P
		Low (n = 184)	High (n = 222)	
pT stage, n (%)	85 (20.9)	41 (22.7)	44 (20.4)	0.65
T0	185 (45.6)	84 (46.4)	101 (46.8)	
T1	93 (22.9)	44 (24.3)	49 (5.7)	
T2	29 (7.1)	11 (6.1)	18 (8.3)	
T3	5 (1.2)	1 (0.6)	4 (1.9)	
T4	9 (2.2)			
Unknown				
pN, n (%)	194 (47.8)	91 (50.6)	103 (47.2)	0.51
Negative	204 (50.2)	89 (49.4)	115 (52.8)	
Positive	8 (2.0)			
Unknown				
ER expression, n (%)	51 (12.6)	24 (17.6)	27 (15.5)	0.62
Negative	259 (63.8)	112 (82.4)	147 (84.5)	
Positive	96 (23.6)			
*Unknown**				
PR expression, n (%)	103 (25.4)	45 (33.1)	58 (33.3)	0.96
Negative	207 (51.0)	91 (66.9)	116 (66.7)	
Positive	96 (23.6)			
*Unknown**				
HER-2 expression, n (%)		0.93		
Negative	254 (62.6)	112 (83.0)	142 (82.6)	
Positive	53 (13.1)	23 (17.0)	30 (17.4)	
*Unknown**	99 (24.4)			

*Unknown includes pathological complete response and missing data. Data are given as counts (percentages).

Characteristic	Entire cohort (n = 406)	Neutrophil-to-lymphocyte ratio		P
		Low (n = 184)	High (n = 222)	
Ki-67 expression, n (%)	208 (51.2)	91 (70.5)	117 (69.2)	0.81
≤ 15%	90 (22.2)	38 (29.5)	52 (30.8)	
> 15%	108 (26.6)			
*Unknown**				
Tumor grade, n (%)	38 (9.4)	20 (14.6)	18 (10.7)	0.57
1	216 (53.2)	95 (69.3)	121 (71.6)	
2	52 (12.8)	22 (16.1)	30 (17.8)	
3	100 (24.6)			
*Unknown**				

*Unknown includes pathological complete response and missing data. Data are given as counts (percentages).

Outcomes

Table 4 shows patients' response to NAC according to the Miller-Payne system in the two NLR groups. No significant intergroup difference was evident (P = 0.20), and the rate of pCR in the low NLR group (22.3%) was similar to that observed in the high NLR group (19.8%). The median follow-up time was 53.3 months (range: 11.4 – 194.4 months). Median DFS and OS were not reached. 12-, 36-, and 60-months disease-free survival rates were 94.6%, 86.1%, 77.8%, respectively. 12-, 36-, and 60-months overall survival rates were 99.8%, 96.3%, 90.3%, respectively. The DFS of patients in the high and low NLR groups was similar (P = 0.091; Fig. 1A). Similarly, the OS of patients in the high and low NLR groups did not differ significantly (P = 0.181; Fig. 1B).

Table 4
Pathological response to neoadjuvant chemotherapy according to the Miller-Payne grading system of the study patients in different neutrophil-to-lymphocyte ratio groups (low *versus* high)

Miller-Payne grade, n (%)	Entire cohort (n = 406)	Neutrophil-to-lymphocyte ratio		P
		Low (n = 184)	High (n = 222)	
1	6 (1.5)	5 (2.7)	1 (0.5)	0.20
2	58 (14.3)	21 (11.4)	37 (31.7)	
3	167 (41.1)	75 (40.8)	92 (41.4)	
4	90 (22.2)	42 (22.8)	48 (20.6)	
5	85 (20.9)	41 (22.3)	44 (19.8)	
Data are given as counts (percentages).				

Discussion

Previous studies have shown that numerous factors – including age, genetic polymorphisms, as well as ER, PR, and HER2 expression status – may be predictive of response to NAC in women with breast cancer[6]. For example, HER2-positive and triple-negative breast malignancies generally show good responses to NAC[6]. However, some of these variables become available only following detailed pathological investigations. There is, therefore, an urgent need for reliable prognostic tools grounded on simple pretreatment variables. In this scenario, some readily available parameters originated from routine complete blood count have been extensively investigated to predict treatment response[7,9]. In general, white blood cell count reflects an individual's systemic and/or local inflammatory status[8]. Neutrophils are known to produce cytokines, chemokines, and growth factors that may promote angiogenesis as well as tumor cell proliferation and migration. In contrast, lymphocytes are responsible for antitumor-specific immune response – including T-lymphocyte tumor infiltration and cytotoxic T-lymphocyte-mediated antitumor activity[8,10]. Starting from these premises, it can be expected that a high NLR – an index that reflects the balance between inflammation and immunoreaction in cancer – would predict a lower response to treatment and less favorable outcomes[9]. However, we did not observe any significant association between pretreatment NLR and pCR to NAC in Turkish women with breast malignancies. Further, NLR was not predictive of DFS and OS in this patient group, although there was a non-significant trend toward a better DFS in patients with low NLR. We cannot exclude that the cutoff value might affect the predictive and prognostic performances of NAC. Notably, such cutoffs may be dependent on the specific patient population and/or ethnicity. The optimal cutoff for NLR in our study was 1.95, being lower than that previously reported by Azab et al.[11] These authors demonstrated that patients with breast

cancer in the highest NLR quartile (> 3.3) had higher 1- and 5-year mortality rates than those in the lowest NLR quartile (< 1.8)[11]. In a study of 215 Chinese women, Chen et al.[12] shown that women who had an NLR > 2.06 before chemotherapy had higher rates of pCR. However, negative results have been published as well. Suppan at al.[13] failed to demonstrate an association between NLR and response to neoadjuvant chemo- or hormone therapy in a study of 247 Austrian women. Similar results have been reported by Erylmaz et al.[14] in research conducted on 78 Turkish women. As described above, the optimal methods of selecting NLR cutoff values remain unclear, and this methodological caveat may yield conflicting results. Further, the potential existence of inflammatory co-morbidities may have confounded the predictive value of NLR and could have played a role in the discrepant findings published in the literature.

Some limitations of our study merit comment, including the retrospective design and the inclusion of women of Turkish descent only. Furthermore, we did not perform subgroup analyses according to breast cancer subtypes.

Based on our results, pretreatment NLR seems to have no significant predictive value in Turkish women with breast cancer who received NAC. However, prospective studies will surely provide deeper insights into the prognostic significance of NLR in this clinical setting.

Materials And Methods

Study patients and variable collection

This is a retrospective analysis of collected data. The study sample consisted of 406 consecutive Turkish women with breast cancer scheduled to undergo NAC before attempting cytoreductive surgery at the Department of Oncology or the Department of General Surgery, Uludag University Medical Center (Bursa, Turkey). The following variables were extracted from clinical records in all participants: age; menopausal status; surgical approach; pre- and post-treatment pathological stage; histology type; pre- and post-treatment tumor stage; pre- and post-treatment axillary lymph node status; pre- and post-treatment expression of estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67; post-treatment tumor grade; use of chemo-, radio- and hormonal therapy; and use of trastuzumab.

The staging of the patients was carried out according to the American Joint Committee on Cancer (AJCC) Staging Manual, eighth edition.
A pretreatment complete blood count was obtained in the week preceding NAC, and the NLR was calculated from absolute counts of neutrophils and lymphocytes.

Ethical statement

The study protocol complied with the tenets of the Helsinki declaration. The Clinical Research Ethics Committee of the Bursa Uludag University Faculty of Medicine approved the study and all exemptions from informed consent due to the retrospective nature (Approval number: 2020-8/43).

Outcomes

The primary outcome measure was pCR to NAC. The Miller-Payne system[15] was used to grade response to NAC, as follows: grade 1, no change or some alteration to individual malignant cells, but no reduction in overall cellularity (pNR); grade 2, minor loss of tumor cells, but overall cellularity still high; up to 30% loss (pPR); grade 3, between an estimated 30% and 90% reduction in tumor cells (pPR); grade 4, marked disappearance of tumor cells such that only small clusters or widely dispersed individual cells remain; 90% loss of tumor cells (almost pCR); and grade 5, no malignant cells identifiable in sections from the site of the tumor; only vascular fibroelastotic stroma remains, often containing macrophages; however, ductal carcinoma in situ may be present (pCR). Secondary outcome measures included disease-free survival (DFS) and overall survival (OS). DFS and OS were calculated as the time (in months) from the date of breast cancer diagnosis to the date of disease recurrence and death, respectively.

Statistical analysis

The optimal cutoff point for NLR was determined using receiver operating characteristic (ROC) curve analysis taking disease recurrence as the endpoint of interest. Patients were divided into two groups (high versus low) according to the optimal cutoff value (NLR = 1.95; low NLR $\leq$ 1.95, high NLR >1.95). The general characteristics of the study patients are presented using descriptive statistics (median, ranges, counts, and percentages). Intergroup differences were assessed with the Mann-Whitney U test (continuous variables) or the chi-square test (categorical variables). Survival curves were plotted using the Kaplan-Meier method and compared with the log-rank test. All calculations were performed with SPSS, version 22.0 (IBM, Armonk, NY, USA). Two-tailed p values <0.05 were considered statistically significant.

Declarations

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COMPETING INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

E.C. conceived the study design, A.B.S, B.O, K.S., and G.Y. collected the data, A.B.S. performed statistical analyses. E.C. wrote the manuscript. T.E., A.D., S.T., M.S.G., and S.C. edited the manuscript.

All authors have read and approved the final manuscript.

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Figures

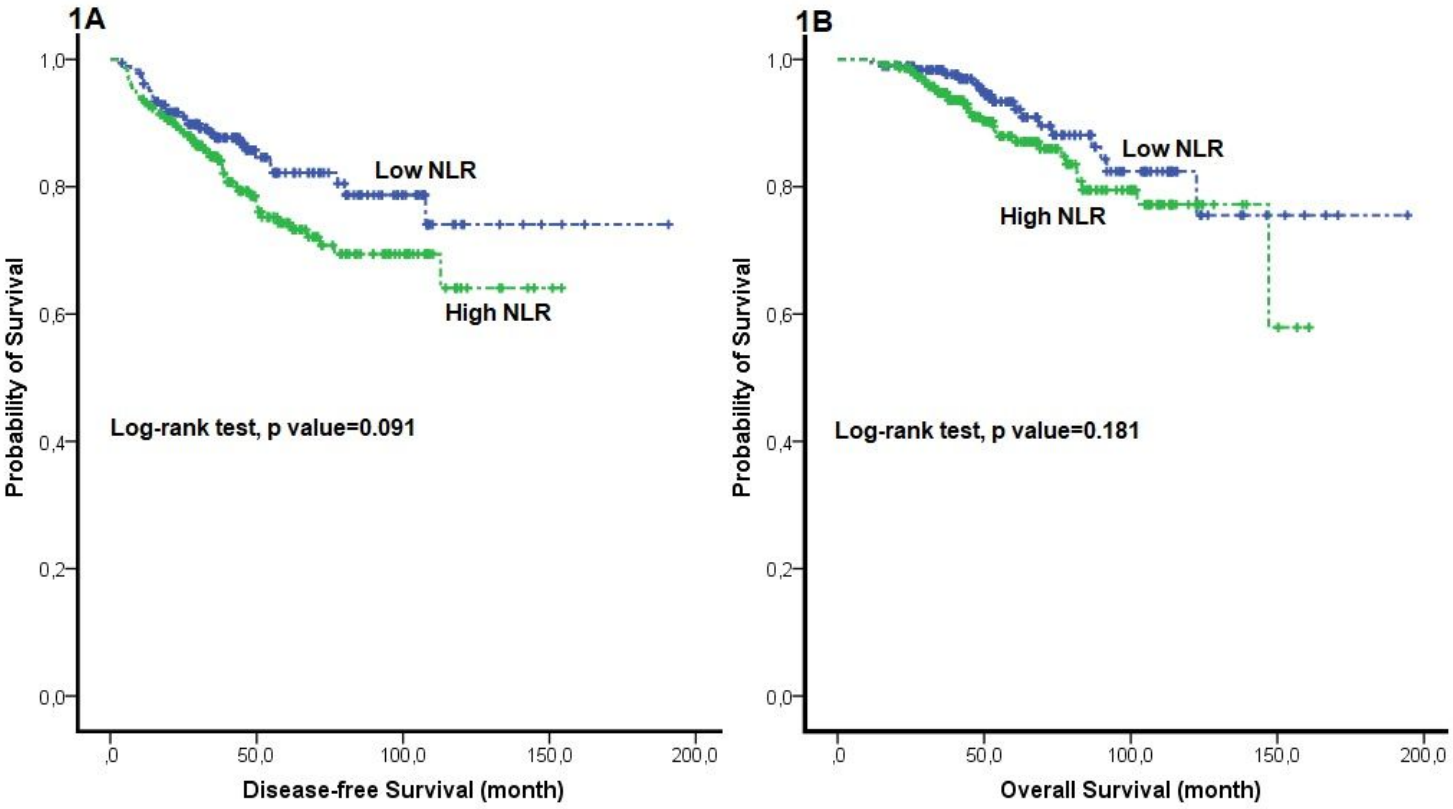

Figure 1

Kaplan-Meier plots of survival endpoints in different neutrophil-to-lymphocyte ratio groups (low versus high): (A) disease-free survival; (B) overall survival.