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Assessment of the Role of Neutrophil-Lymphocyte Ratio as a Prognostic Biomarker in Breast Cancer Patients: A Cross-sectional Study

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Abstract

Background/Aim: Peripheral blood neutrophil–lymphocyte ratio (NLR) has been reported to predict the effects of surgery and chemotherapy in breast cancer patients. However, the majority of the studies performed only one-time evaluation before commencement of treatment, while few evaluated the ratio over a period of time. In this study, we calculated NLR before surgery and postoperative adjuvant chemotherapy for patients with resectable breast cancer who underwent surgery as the initial treatment, and examined its correlation with clinicopathological factors and prognosis.

Patients and Methods: A total of 1,095 patients with primary resectable breast cancer underwent curative resection as the first line of treatment between December 2007 and October 2018. Of these 1,095 patients, 178 were included in this study. Peripheral blood was collected before, and after the surgery. Preoperative NLR was evaluated during the first hospital visit before biopsy. Postoperative NLR was evaluated using peripheral blood collected immediately prior to postoperative adjuvant chemotherapy. The cut-off value of NLR was set to 3, which has been reported to be the most commonly used value.

Results: Examination of postoperative NLR and prognosis in 24 breast cancer patients with higher pre-NLR revealed no significant difference [disease-free survival (DFS), p=0.320; overall survival (OS), p=0.409, log-rank test]. However, when post-NLR and prognosis were examined in 154 breast cancer patients with lower pre-NLR, the lower post-NLR group showed significant prolongation in DFS (p<0.001, log-rank test). Furthermore, OS tended to be prolonged in the lower post-NLR group (p=0.056, log-rank test). Multivariate analysis of DFS in 154 breast cancer patients with lower pre-NLR showed that large tumors [hazard ratio (HR)=4.132, p=0.009], nuclear grade 3 (HR=2.746, p=0.043), and higher post-NLR (HR=4.639, p=0.003) were independent factors.

Conclusion: Prognosis of breast cancer patients can be predicted by evaluating the NLR over time.

Keywords: Breast cancer, chemotherapy, NLR, prognostic marker, tumor immunology.

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Introduction

Previous meta-analyses have reported that peripheral blood neutrophil–lymphocyte ratio (NLR) predicts the effects of surgery and chemotherapy in breast cancer patients (1, 2). Neutrophils induce tumor cell proliferation *via* various ligands and cytokines (3-5), as opposed to lymphocytes that suppress cancer progression (6). It has been reported that NLR can evaluate tumor immune status in the host. However, neutrophil and lymphocyte counts can also be easily affected by subclinical trauma and infection (7). Nevertheless, the majority of the studies performed only one-time evaluation of NLR before commencement of the treatment, while few of them evaluated the ratio over a period of time.

Therefore, we hypothesized that measuring NLR twice, before and after surgery could predict the therapeutic effect more sensitively. In this study, we calculated NLR before surgery and postoperative adjuvant chemotherapy for patients with resectable breast cancer who underwent surgery as the initial treatment, and examined its correlation with clinicopathological factors and prognosis.

Patients and Methods

Study population and patient background. A total of 1,095 patients with primary resectable breast cancer who underwent curative surgery as the initial treatment at Osaka City University Hospital (currently Osaka Metropolitan University Hospital) between December 2007 and October 2018 were included in this study (Figure 1). All patients were histopathologically diagnosed with breast cancer using core needle biopsy (CNB) or vacuum-assisted biopsy (VAB) before treatment. Breast cancer staging was evaluated using ultrasonography (US), computed tomography (CT), and bone scintigraphy. Feasibility of radical resection was decided based on these results, and curative surgery was performed. Mastectomy or breast-conservation surgery was performed for the primary lesion. Axillary lymph node dissection (ALND) was performed in patients diagnosed with axillary lymph node metastasis using preoperative imaging. Intraoperative sentinel lymph node biopsy (SLNB) was performed in patients who did not show evidence of axillary lymph node metastasis on preoperative imaging. Sentinel lymph node was identified using the combination of radioisotope and dye methods (8, 9). Pathological evaluation of metastases to the sentinel lymph node was performed in accordance with a previously reported method of sectioning the lymph node into 2 mm slices (10, 11). On the basis of the pathological results of SLNB, ALND was performed when necessary.

Pathological infiltration and malignancy of surgically resected specimens were evaluated. Expression of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and Ki67 was examined using immunohistochemical staining. We defined ER-positive and/or PgR positive/HER2 negative breast cancer as HR+HER2-BC; ER-positive and/or PgR positive/HER2 positive breast cancer as HR+HER2+BC; ER negative/PgR negative/HER2 positive breast cancer as hormone receptor (HR)-HER2+BC; and ER negative/PgR negative/HER2 negative breast cancer as triple-negative breast cancer (TNBC). Cutoff for Ki-67 was set to 20% in accordance with previous reports (12). Lymphatic and venous invasions were evaluated using immunohistochemical staining.

Need for postoperative adjuvant chemotherapy and chemotherapeutic regimen were selected by the attending physician based on the degree of cancer progression and pathological findings. Two main types of postoperative adjuvant chemotherapy regimens were selected for this study. The first regimen consisted of four courses of FEC100 (500 mg/m^2 fluorouracil, 100 mg/m^2 epirubicin, and 500 mg/m^2 cyclophosphamide) every three weeks, followed by 12 courses of 80 mg/m^2 paclitaxel administered weekly. The second regimen comprised four courses of TC75 (75 mg/m^2 docetaxel and 600 mg/m^2 cyclophosphamide) every three weeks. For HER2-positive breast cancer, trastuzumab was administered in combination with paclitaxel or docetaxel/cyclophosphamide. All patients received standard postoperative adjuvant radiation and

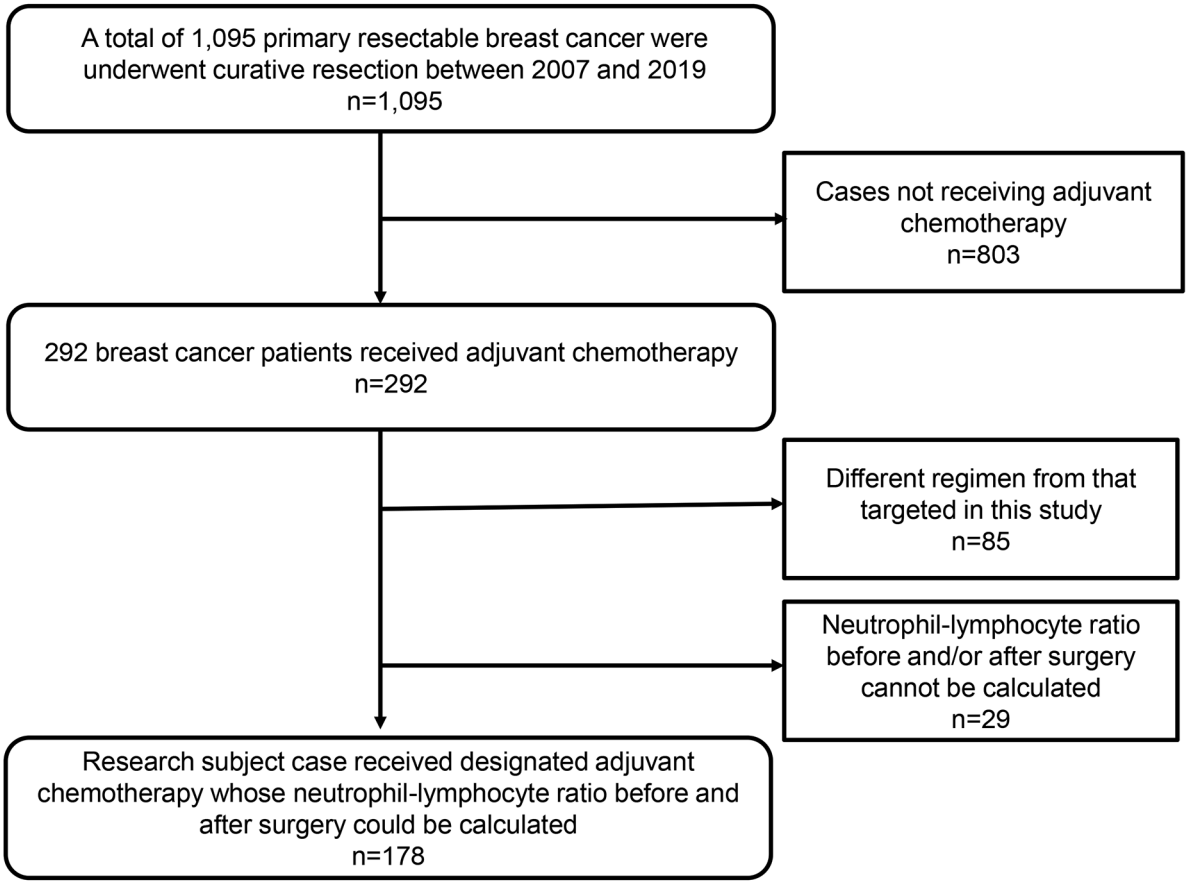

Figure 1. *Consort diagram. A total of 1,095 patients with primary resectable breast cancer underwent curative resection between December 2007 and October 2018 at Osaka City University Hospital. Of these, 292 patients received adjuvant chemotherapy. Eighty-five patients were excluded because they received a different chemotherapeutic regimen, and another 29 patients were excluded because their pre- or post-operative peripheral blood neutrophil–lymphocyte ratio (NLR) could not be calculated. As a result, the remaining 178 patients formed the final study population.*

endocrine therapy, as required, after postoperative adjuvant chemotherapy. The median follow-up time was 2,102 days (range=60-4,889 days).

Blood sample analysis. Peripheral blood was collected before and after the surgery. Preoperative NLR (named "pre-NLR") was evaluated during the first hospital visit before biopsy. Postoperative NLR (named "post-NLR") was evaluated using peripheral blood collected immediately prior to postoperative adjuvant chemotherapy. The number of absolute leukocytes was determined using a hemocytometer. The absolute neutrophil and lymphocyte counts were measured by determining the percentages of different cell types using a Coulter LH 750 Hematology Analyzer (Beckman Coulter, Brea, CA, USA). NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The cut-off value of NLR was set to 3, which is the most commonly reported value (1).

Of the 207 patients, 29 were excluded from the study since their pre or postoperative NLR values could not be calculated. The study participants were divided into four groups to assess whether the results were higher or lower than the cutoff values before and after the surgery. In this study, we defined four groups: pre-NLR and post-NLR evaluations were indicated by the first and second letters,

respectively, with "H" being higher than the cutoff value and "L" being lower than the cutoff value. For example, patients with higher pre-NLR and lower post-NLR belonged to the "HL-NLR group".

Statistical analysis. All statistical analyses were performed using JMP software package (SAS, Tokyo, Japan). Relationship between each factor was examined using χ^2 test. Kaplan–Meier method and log-rank test were used for comparison between disease-free survival (DFS) and overall survival (OS). Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using the Cox proportional hazards model. Multivariate analysis was performed using the Cox regression model. Statistical significance was set at p-value <0.05.

Results

Clinicopathological features. Of the 1,095 patients who underwent surgery as initial treatment, 178 were included in this study. Table I shows the clinicopathological features of the patients. The median age was 56 (range=28-79) years and the median tumor size was 20.9 mm (range=3.1-73.8 mm). Seventy-seven patients (43.3%) underwent SLNB but did not show any evidence of metastases. ALND was omitted in nine patients (5.0%) since only micro metastases were found by SLNB, while the procedure was performed in 92 (51.6%) patients. All surgical complications were Grade II or lower in the Clavien-Dindo Classification, and were completely resolved before the commencement of adjuvant chemotherapy. Positive expression of ER, PgR and HER2 was found in 117 (65.7%), 70 (39.3%), and 42 (23.6%) patients, respectively. As a result, 97 patients (54.4%), accounting for about half of the participants, had HR+HER2-BC, while 39 patients (21.9%) had TNBC, and only 18 patients (10.1%) had HR-HER2+BC. Eighty patients (44.9%) showed increased Ki67 expression. Breast-conservation surgery was performed in 76 patients (42.7%), all of whom received postoperative radiation therapy for residual mammary gland after postoperative adjuvant chemotherapy. Pathological examination of the surgical specimens revealed lymphatic and venous infiltrations in 96 (53.9%) and 31 (17.4%) patients, respectively. Eighty-five (47.8%) patients were diagnosed with nuclear grade 3. Adjuvant chemotherapy was initiated one–two months after surgery, when the wound had healed completely. FEC followed weekly paclitaxel (± trastuzumab) regimen in 93 patients (52.2%) and TC (± trastuzumab) regimen in 85 patients (47.8%).

Median pre-NLR was 1.91 (range=0.42-11.51), and 24 patients (13.5%) were categorized into the high pre-NLR group according to the defined cutoff value. Median post-NLR was 1.91 (range=0.42-10.43), and 33 patients (18.5%) were classified into the high post-NLR group according to the defined cutoff value. Twelve (6.7%) patients had higher NLR values both before and after surgery, and belonged to the so-called HH-NLR group. Correlations between breast cancer subtypes and clinicopathological factors were investigated.

Correlation between NLR and clinicopathological factors. After stratifying patients according to preoperative NLR, we examined whether postoperative NLR correlated with clinicopathological factors (Table II). Patients in the HL-NLR group had more HR-HER2+BC (p=0.028) and higher Ki67 (p=0.041) than those in the HH-NLR group. No correlation was found between postoperative NLR and clinicopathologic factors in patients with a lower preoperative NLR.

Prognosis prediction by NLR. When divided into four groups according to NLR before and after surgery, significant differences were found in DFS and OS (p<0.001, log-rank, respectively) (Figure 2). Examination of postoperative NLR and prognosis in 24 breast cancer patients with higher pre-NLR revealed no significant difference (DFS, p=0.320; OS, p=0.409; log-rank test). However, when post-NLR and prognosis were examined in 154 breast cancer patients with lower pre-NLR, the lower post-NLR group showed significant prolongation of DFS (p<0.001, log-rank). Furthermore, OS tended to be

Table I. *Clinicopathological features of 178 breast cancer patients receiving adjuvant chemotherapy.*

Parameters	Number of all patients (n=178) (%)
Age at operation (years old)	Median 56 (range=28-79)
Tumor size (mm)	Median 20.9 (range=3.1-73.8)
Number of pathological lymph node metastasis	77 (43.2%)/9 (5.0%)/51 (28.7%)/
0/micrometastasis/1-3/4-9/≥10	27 (15.2%)/14 (7.9%)
Estrogen receptor	61 (34.3%)/117 (65.7%)
Negative/Positive	
Progesterone receptor	108 (60.7%)/70 (39.3%)
Negative/Positive	
HER2	136 (76.4%)/42 (23.6%)
Negative/Positive	
Ki67	98 (55.1%)/80 (44.9%)
≤20%/>20%	
Intrinsic subtype	97 (54.5%)/24 (13.5%)/
HR+HER2-BC/HR+HER2+BC/HR-HER2+BC/TNBC	18 (10.1%)/39 (21.9%)
Surgical treatment	76 (42.7%)/102 (57.3%)
Breast conserving treatment/Mastectomy	
Treatment of axillary lymph nodes	86 (48.3%)/92 (51.7%)
SLNB only/ALND	
Lymphatic invasion	82 (46.1%)/96 (53.9%)
ly0/ly1	
Venous invasion	147 (82.6%)/31 (17.4%)
v0/v1	
Nuclear grade	36 (20.2%)/71 (39.9%)/71 (39.9%)
1/2/3	
Adjuvant chemotherapy	93 (52.2%)/85 (47.8%)
FEC followed weekly paclitaxel (± Trastuzumab)/TC (± Trastuzumab)	
Preoperative NLR	Median 1.91 (range, 0.42-11.51)
Low/High	154 (86.5%)/24 (13.5%)
Postoperative NLR	Median 1.91 (range, 0.42-10.43)
Low/High	145 (81.5%)/33 (18.5%)

HER2: Human epidermal growth factor receptor 2; HR: hormone receptor; HR+HER2-BC: hormone receptor-positive and HER2 negative breast cancer (ER+ and/or PgR+, and HER2-); HR+HER2+BC: hormone receptor-positive and HER2 positive breast cancer (ER+ and/or PgR+, and HER2+); HR-HER2+BC: hormone receptor-negative and HER2 positive breast cancer (ER-, PgR-, and HER2+); TNBC: triple negative breast cancer (ER-, PgR-, and HER2-); SLNB: sentinel lymph node biopsy; ALND: axillary lymph node dissection; FEC: fluorouracil, epirubicin, cyclophosphamide; TC: docetaxel, cyclophosphamide; NLR: neutrophil to lymphocyte ratio.

prolonged in the lower post-NLR group (p=0.056, log-rank). Multivariate analysis of DFS in 154 breast cancer patients with lower pre-NLR showed that large tumors (HR=4.132; p=0.009), nuclear grade 3 (HR=2.746; p=0.043), and higher post-NLR (HR=4.639, p=0.003) were independent prognostic factors (Table III). Univariate analysis of OS showed no significant association with higher post-NLR (HR=4.866; p=0.114), while multivariate analysis identified association high Ki67 expression (HR=uncalculated, p=0.007) as an independent prognostic factor (Table IV).

Discussion

Monitoring the immune environment surrounding cancer, which affects cancer progression and metastasis, may help predict the effects and prognosis of surgery and chemotherapy. In recent years, drugs that control the tumor immune microenvironment, such as atezolizumab, are being used in clinical practice. However, surgical removal of the tumor is required for histopathological examination. Furthermore, if surgery is selected as the initial treatment, subsequent

Table II. *Correlation between postoperative neutrophil to lymphocyte ratio (NLR) and clinicopathological features after dividing patients according to preoperative NLR.*

Parameters	Low preoperative NLR patients (n=154)			High preoperative NLR patients (n=24)		
	LL-NLR group (n=133)	LH-NLR group (n=21)	*p*-Value	HL-NLR group (n=12)	HH-NLR group (n=12)	*p*-Value
Age (years old)			0.245			1.000
≤60	84 (63.2%)	16 (52.4%)		8 (66.7%)	8 (66.7%)	
>60	49 (36.8%)	5 (47.6%)		4 (33.3%)	4 (33.3%)	
Tumor size (mm)			0.817			0.673
≤20.0	66 (49.6%)	11 (52.4%)		4 (33.3%)	5 (41.7%)	
>20.0	67 (50.4%)	10 (47.6%)		8 (66.7%)	7 (58.3%)	
Pathological lymph node metastasis			0.765			1.000
Negative (Including micrometastasis)	68 (51.1%)	10 (47.6%)		4 (33.3%)	4 (33.3%)	
Positive	65 (48.9%)	11 (52.4%)		8 (66.7%)	8 (66.7%)	
Estrogen receptor			0.502			0.178
Negative	48 (36.1%)	6 (28.6%)		5 (41.7%)	2 (16.7%)	
Positive	85 (63.9%)	15 (71.4%)		7 (58.3%)	10 (83.3%)	
Progesterone receptor			0.794			1.000
Negative	80 (60.2%)	12 (57.1%)		8 (66.7%)	8 (66.7%)	
Positive	53 (39.8%)	9 (42.9%)		4 (33.3%)	4 (33.3%)	
HER2			0.665			0.178
Negative	102 (76.7%)	17 (81.0%)		7 (58.3%)	10 (83.3%)	
Positive	31 (23.3%)	4 (19.0%)		5 (41.7%)	2 (16.7%)	
Ki67			0.221			0.041
≤20%	76 (57.1%)	9 (42.9%)		4 (33.3%)	9 (75.0%)	
>20%	57 (42.9%)	12 (57.1%)		8 (66.7%)	3 (25.0%)	
Intrinsic subtype			0.428			0.408
Not HR+HER2-BC	63 (47.4%)	8 (38.1%)		6 (50.0%)	4 (33.3%)	
HR+HER2-BC	70 (52.6%)	13 (61.9%)		6 (50.0%)	8 (66.7%)	
Intrinsic subtype			0.941			0.028
Not HR-HER2+BC	121 (91.0%)	19 (90.5%)		8 (66.7%)	12 (100.0%)	
HR-HER2+BC	12 (9.0%)	2 (9.5%)		4 (33.3%)	0 (0.0%)	
Intrinsic subtype			0.614			0.537
Not TNBC	101 (75.9%)	17 (81.0%)		11 (91.7%)	10 (83.3%)	
TNBC	32 (24.1%)	4 (19.0%)		1 (8.3%)	2 (16.7%)	
Surgical treatment			0.103			0.218
Breast conserving treatment	51 (38.3%)	12 (57.1%)		5 (41.7%)	8 (66.7%)	
Mastectomy	82 (61.7%)	9 (42.9%)		7 (58.3%)	4 (33.3%)	
Lymphatic invasion			0.864			1.000
ly0	66 (49.6%)	10 (47.6%)		3 (25.0%)	3 (25.0%)	
ly1	67 (50.4%)	11 (52.4%)		9 (75.0%)	9 (75.0%)	
Venous invasion			0.568			0.083
v0	114 (85.7%)	17 (81.0%)		6 (50.0%)	10 (83.3%)	
v1	19 (14.3%)	4 (19.0%)		6 (50.0%)	2 (16.7%)	
Nuclear grade			0.694			0.219
1, 2	82 (61.7%)	12 (57.1%)		5 (41.7%)	8 (66.7%)	
3	51 (38.3%)	9 (42.9%)		7 (58.3%)	4 (33.3%)	
Adjuvant chemotherapy			0.608			0.132
FEC followed weekly paclitaxel (± Trastuzumab)	65 (48.9%)	9 (42.9%)		8 (66.7%)	11 (91.7%)	
TC (± Trastuzumab)	68 (51.1%)	12 (57.1%)		4 (33.3%)	1 (8.3%)	

HER2: Human epidermal growth factor receptor 2; HR: hormone receptor; HR+HER2-BC: hormone receptor-positive and HER2 negative breast cancer (ER+ and/or PgR+, and HER2-); HR-HER2+BC: hormone receptor-negative and HER2 positive breast cancer (ER-, PgR-, and HER2+); TNBC: triple negative breast cancer (ER-, PgR-, and HER2-); FEC: fluorouracil, epirubicin, cyclophosphamide; TC: docetaxel, cyclophosphamide.

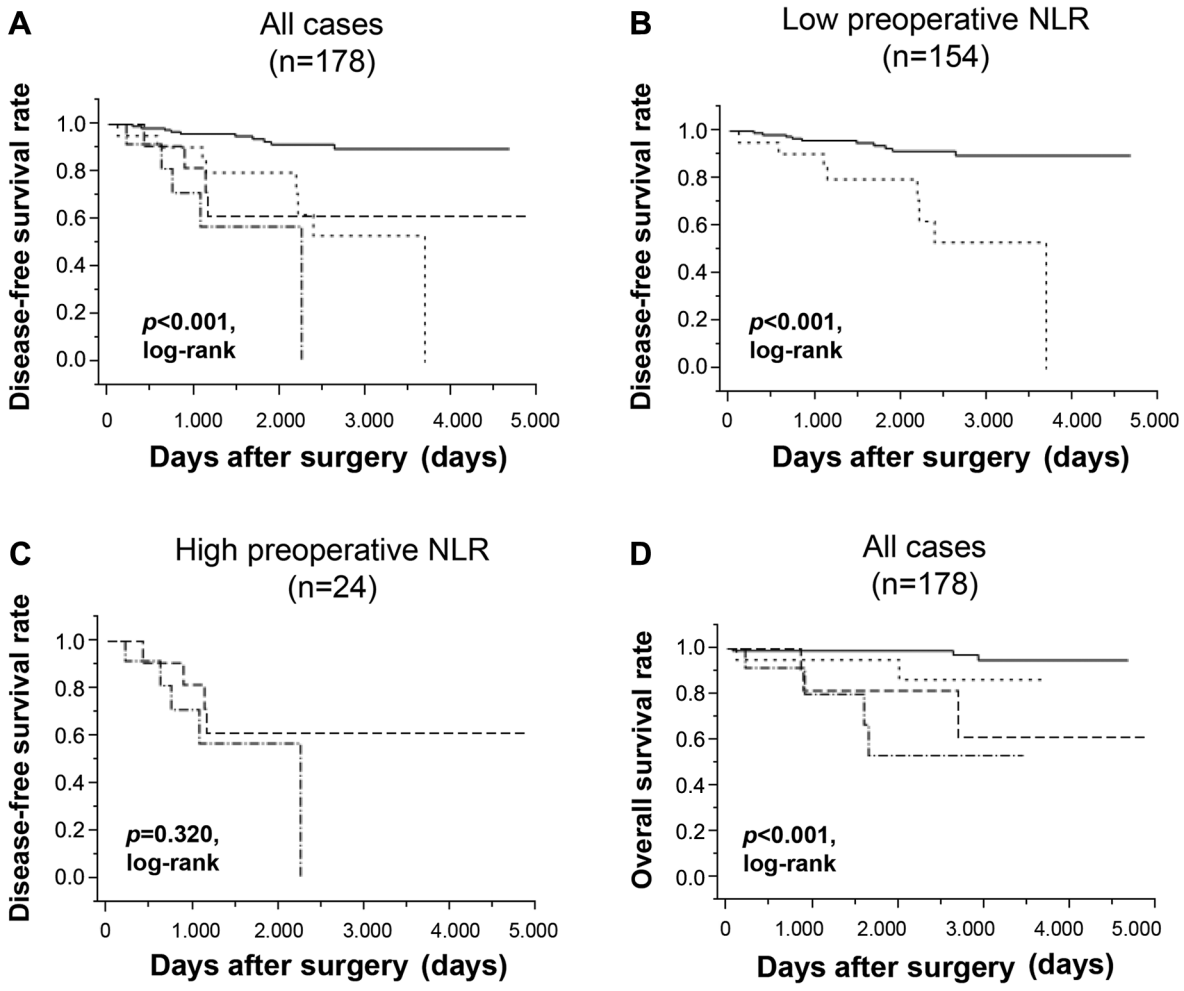

Figure 2. *Continued*

evaluation over time is not possible. Contrarily, NLR, an index that reflects the balance between the host's tumor-promoting environment and anti-tumor immune status, is relatively easy to evaluate over time. However, NLR is susceptible to alterations by subclinical infections, trauma, and other diseases. To the best of our knowledge, ours is the first study to have evaluated NLR over time and in a complex manner, both before and after surgery in breast cancer patients.

A meta-analysis reported that NLR showed no correlation with clinical pathological factors such as progression in breast cancer patients (2). In our study, a correlation was found between pre-NLR and the selected adjuvant chemotherapy regimen, which was determined by the attending physician. Therefore, this correlation is unlikely to have influenced pre-NLR. In other words, the fact that no correlation was found between pre-NLR and clinicopathological factors in this study is consistent with the findings of the above-mentioned meta-analysis. There were concerns regarding the effects of surgical inflammation on post-NLR. Although there are no specific reports regarding the exact time when inflammation due to surgical wound healing ceases, it has been reported that one month after surgery is an

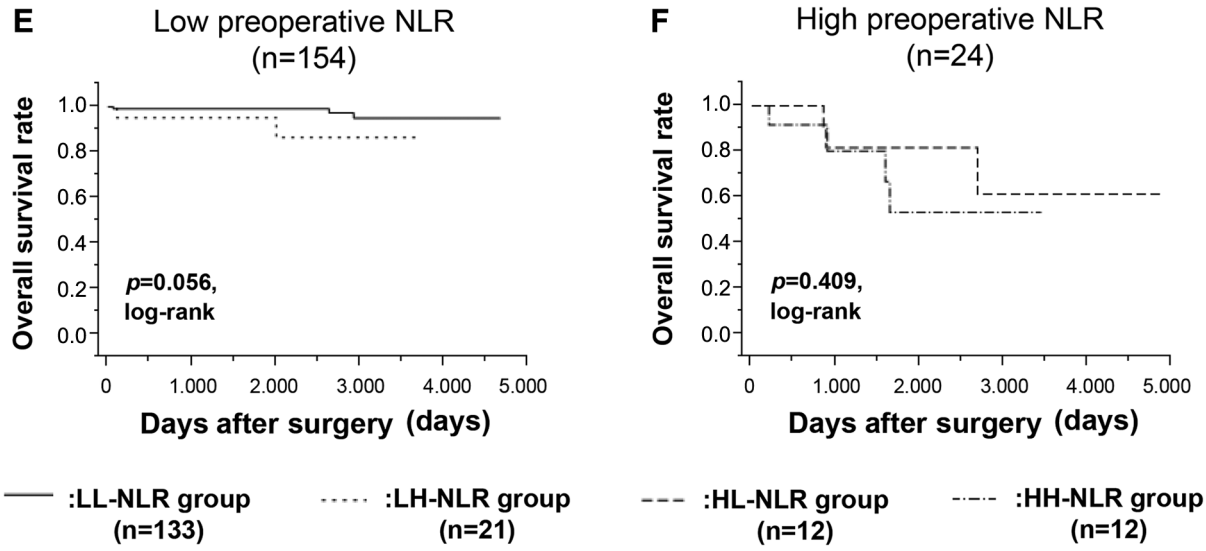

Figure 2. *Kaplan-Meier stratification curve for disease-free survival (DFS) and overall survival (OS) based on four groups divided by peripheral blood neutrophil–lymphocyte ratio (NLR) before and after surgery. DFS for all cases (p<0.001, log-rank) (A). DFS for patients with low preoperative NLR (p<0.001, log-rank) (B). DFS for patients with high preoperative NLR (p=0.320, log-rank) (C). OS for all cases (p<0.001, log-rank) (D). OS for patients with low preoperative NLR (p=0.056, log-rank) (E). OS for patients with high preoperative NLR (p=0.409, log-rank) (F).*

appropriate time point for assessment in lung and colorectal cancer (13, 14). Thus, it was considered that blood sampling before adjuvant chemotherapy was not affected by inflammation due to surgery, since surgery for breast cancer is comparatively less invasive than surgeries for lung or colorectal cancer. The fact that no significant difference was observed between pre and postoperative NLR indicated that surgical invasion and macroscopic tumor elimination from the patient did not affect NLR. As a result, post-NLR was also unaffected by other clinicopathological factors. However, the partial correlation of post-NLR with clinicopathological factors, after dividing patients into two groups based on pre-NLR, was not clearly established.

Tumor size, generally regarded as a prognostic factor, affected the overall prognosis in this study, but was not associated with prognosis in relation to lymph node metastasis or subtype. The reason for this finding may be the selection of patients who received adjuvant chemotherapy. Patients with TNBC or HR-HER2+BC were included in this study because they required adjuvant chemotherapy, even at a relatively early stage. In contrast, in patients with HR+HER2-BC, postoperative endocrine therapy can prevent recurrence; therefore, adjuvant chemotherapy was limited to highly advanced cases. Even if NLR was less susceptible to clinicopathological factors, attention should be paid to the prognostic analysis results of this study.

Higher pre-NLR led to poor DFS and OS (1, 2). Similarly, higher post-NLR was also associated with poor DFS and OS. In this study, wherein participants were divided into four groups according to pre and postoperative NLR, it was observed that higher NLR before and/or after surgery was associated with poor prognosis. In other words, patients with higher post-NLR had a poor prognosis similar to those with higher pre-NLR, even when their pre-NLR values were initially low. To the best of our knowledge, this is the first report to examine pre and postoperative NLR in breast cancer, but similar studies have been done in gastric cancer (15), hepatocellular carcinoma (16, 17), and colorectal cancer (18, 19). Several studies have reported that a persistently elevated inflammatory status can impair anti-

Table III. *Univariate and multivariate analysis of disease-free survival for 154 breast cancer patients with low preoperative neutrophil to lymphocyte ratio (NLR) receiving adjuvant chemotherapy.*

	Univariate analysis			Multivariate analysis		
Parameters	Hazard ratio	95%CI	*p*-Value	Hazard ratio	95%CI	*p*-Value
Age (years old) ≤60 *vs*. >60	1.537	0.586-3.904	0.371			
Tumor size (mm) ≤20.0 *vs*. >20.0	4.543	1.621-16.083	0.003	4.132	1.394-15.285	0.009
Pathological lymph node metastasis Negative *vs*. Positive	1.191	0.462-3.152	0.716			
Estrogen receptor Negative *vs*. Positive	1.130	0.434-3.268	0.808			
Progesterone receptor Negative *vs*. Positive	0.858	0.311-2.202	0.753			
HER2 Negative *vs*. Positive	0.489	0.077-1.729	0.298			
Ki67 ≤20% *vs*. >20%	3.139	1.213-9.056	0.018	1.614	0.556-5.002	0.380
Intrinsic subtype Not HR+HER2-BC *vs*. HR+HER2-BC	1.254	0.492-3.420	0.638			
Intrinsic subtype Not HR-HER2+BC *vs*. HR-HER2+BC	0.610	0.034-2.990	0.606			
Intrinsic subtype Not TNBC *vs.* TNBC	1.215	0.388-3.243	0.717			
Surgical treatment Breast conserving treatment *vs.* Mastectomy	2.047	0.770-6.392	0.155			
Lymphatic invasion ly0 *vs.* ly1	0.885	0.345-2.271	0.797			
Venous invasion v0 *vs.* v1	1.121	0.175-4.056	0.882			
Nuclear grade 1, 2 *vs.* 3	2.779	1.083-7.660	0.034	2.746	1.032-7.978	0.043
Adjuvant chemotherapy FEC followed weekly paclitaxel (± Trastuzumab) *vs*. TC (± Trastuzumab)	1.976	0.749-5.855	0.172			
Postoperative NLR Low *vs*. High	5.738	2.185-14.591	0.001	4.639	1.709-12.253	0.003

NLR: Neutrophil to lymphocyte ratio; CI: confidence interval; HER2: human epidermal growth factor receptor 2; HR: hormone receptor; HR+HER2-BC: hormone receptor-positive and HER2 negative breast cancer (ER+ and/or PgR+, and HER2-); HR-HER2+BC: hormone receptor-negative and HER2 positive breast cancer (ER-, PgR-, and HER2+); TNBC: triple negative breast cancer (ER-, PgR-, and HER2-); FEC: fluorouracil, epirubicin, cyclophosphamide; TC: docetaxel, cyclophosphamide.

tumor immune responses even after surgical resection (16-18). In breast cancer, some studies have evaluated the changes during chemotherapy (20) one and a half years after the end of treatment (21), after first-line treatment (22), and during recurrence (23). Even in these studies, an increase in NLR or a persistently high NLR was associated with poor prognosis. Some studies have also reported that follow-up NLR, rather than pretreatment NLR, was an independent prognostic factor for late recurrence (21, 24). These studies, including our study, suggest the importance of capturing changes over time, because post-treatment changes may affect the expression of disseminated metastatic cells (25).

Study limitations. The primary limitation of this study was the considerable variation in the degree of cancer

Table IV. *Univariate and multivariate analysis of overall survival for 154 breast cancer patients with low preoperative neutrophil to lymphocyte ratio (NLR) receiving adjuvant chemotherapy.*

	Univariate analysis			Multivariate analysis		
Parameters	Hazard ratio	95%CI	*p*-Value	Hazard ratio	95%CI	*p*-Value
Age (years old) ≤60 *vs*. >60	1.266	0.167-7.640	0.798			
Tumor size (mm) ≤20.0 *vs*. >20.0	2.033	0.332-15.599	0.435			
Pathological lymph node metastasis Negative *vs*. Positive	0.729	0.096-4.402	0.727			
Estrogen receptor Negative *vs*. Positive	0.969	0.160-7.392	0.973			
Progesterone receptor Negative *vs*. Positive	0.964	0.127-5.830	0.968			
HER2 Negative *vs*. Positive	-	-	0.161			
Ki67 ≤20% *vs*. >20%	-	-	0.003	-	-	0.007
Intrinsic subtype Not HR+HER2-BC *vs*. HR+HER2-BC	1.225	0.202-9.316	0.824			
Intrinsic subtype Not HR-HER2+BC *vs*. HR-HER2+BC	-	-	0.345			
Intrinsic subtype Not TNBC vs. TNBC	1.793	0.235-10.874	0.534			
Surgical treatment Breast conserving treatment *vs*. Mastectomy	1.257	0.207-9.594	0.802			
Lymphatic invasion ly0 *vs*. ly1	1.408	0.233-10.694	0.705			
Venous invasion v0 *vs*. v1	-	-	0.345			
Nuclear grade 1, 2 *vs*. 3	2.395	0.396-18.200	0.333			
Adjuvant chemotherapy FEC followed weekly paclitaxel (± Trastuzumab) *vs*. TC (± Trastuzumab)	3.415	0.504-66.811	0.224			
Postoperative NLR Low *vs*. High	4.866	0.639-29.487	0.114	2.682	0.350-16.349	0.306

CI: Confidence interval; HER2: human epidermal growth factor receptor 2; HR: hormone receptor; HR+HER2-BC: hormone receptor-positive and HER2 negative breast cancer (ER+ and/or PgR+, and HER2-); HR-HER2+BC: hormone receptor-negative and HER2 positive breast cancer (ER-, PgR-, and HER2+); TNBC: triple negative breast cancer (ER-, PgR-, and HER2-); FEC: fluorouracil, epirubicin, cyclophosphamide; TC: docetaxel, cyclophosphamide.

progression among different breast cancer subtypes, which may have influenced the observed associations between NLR and prognosis. Another limitation was that few patients had a higher NLR before or after surgery.

Conclusion

Assessing changes in NLR is a method that can be performed relatively easily in clinical settings; therefore, making it highly useful. Pretreatment NLR may be a sensitive indicator for prognosis prediction; however, prognostic accuracy can be improved by evaluating subsequent changes in NLR over time.

Conflicts of Interest

The Authors declare that they have no competing interests in relation to this study.

Authors' Contributions

All Authors were involved in the preparation of this manuscript. KT collected the data, and wrote the manuscript. MN, CW, YT, KO, MS, HK and TM performed the operation and designed the study. SK made substantial contribution to the study design. All Authors read and approved the final manuscript.

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Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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