Original Article

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Neutrophil to Lymphocyte Ratio after Treatment Completion as a Potential Predictor of Survival in Patients with Triple-Negative Breast Cancer

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Conflict of interest

The authors declare that they have no
competing interests.

ABSTRACT

Purpose: Triple-negative breast cancer (TNBC) has been associated with worse prognosis, and biomarkers are needed to identify high-risk patients who may benefit from clinical trials or escalated treatment after completion of standard treatment. We aimed to assess whether the post-treatment neutrophil-to-lymphocyte ratio (NLR) can reflect patient prognosis and determine the follow-up period that can provide the most feasible data.
Methods: In this retrospective analysis involving patients with TNBC, clinicopathological data, including those on peripheral complete blood cell count, were collected. The prognostic powers of serial NLRs obtained at baseline and after treatment completion were compared. Kaplan-Meier curves were generated to compare the overall survival (OS) and distant disease-free survival (DDFS).
Results: In total, 210 patients were enrolled. Forty-three (20.5%) events were detected. Two-thirds of the events (29/43) were related to breast cancer. Most recurrent breast cancer-related diseases (27/29) were detected within 5 years of the initial diagnosis. In contrast, half of the events due to secondary malignancies or non-breast-related diseases (7/14) occurred 5 years after the initial diagnosis. Comparison of the prognostic performance of NLRs at baseline and at 6, 12, and 24 months after treatment completion revealed the strongest prognostic performance at 6 months after treatment completion (area under the curve = 0.745). The high NLR group (NLR >2.47) showed worse OS ($p = 0.006$) and DDFS ($p < 0.001$) than low NLR group.
Conclusion: Elevated post-treatment NLR was significantly associated with worse survival in patients with TNBC. We believe that it can be a useful surrogate marker for identifying high-risk patients with TNBC.

Keywords: Lymphocytes; Neutrophils; Survival; Triple negative breast neoplasms

INTRODUCTION

Triple-negative breast cancer (TNBC) is frequently associated with early recurrence and high mortality [1,2]. However, patients with TNBC are exposed to chemotherapy for a relatively short period, even though their prognosis is worse than that of the patients with other subtypes, such as estrogen receptor (ER)-positive and/or progesterone receptor (PR)-positive

Author Contributions
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breast cancer (requiring 5–10 years of hormonal treatment) and human epidermal growth factor 2 (HER2)-positive breast cancer (requiring 1 year of HER2-targeted treatment) [3-5]. A recent trial involving escalated adjuvant treatment with capecitabine showed meaningful extension of survival with escalated treatment after completion of the standard treatment, which was eventually implemented in the updated treatment guidelines [6]. Therefore, a feasible biomarker for identifying patients with TNBC who can benefit from escalated treatment is necessary.

Factors that are complementary to conventional risk factors, such as lymph node status, are needed for the precise prediction of patient survival. Patients with TNBC have a lower rate of nodal metastasis than those with other subtypes; however, they have a worse prognosis even after adjusting for tumor size and/or nodal stage [7]. Pathological complete remission after neoadjuvant treatment can also provide meaningful information regarding survival, but not all patients undergo preoperative systemic treatment [7]. Therefore, we aimed to determine whether the neutrophil-to-lymphocyte ratio (NLR) after treatment completion can predict the prognosis of patients with TNBC.

METHODS

Study cohort
This retrospective cohort study involved female patients diagnosed with primary TNBC between January 2000 and June 2017 who completed all phases of treatment at Wonju Severance Hospital, Yonsei University, Wonju, Korea.

Tumors were defined as TNBC when ER, PR, and HER2 statuses extracted from the pathology reports were negative based on the American Society of Clinical Oncology/ College of American Pathologists (ASCO/CAP) 2010 and 2013 guidelines [8,9]. Tumors were classified as ER- and PR-negative if they showed < 10% reactivity until 2010; after June 2010, the threshold for ER and PR positivity was decreased to < 1% [8]. HER2 status was assessed using immunohistochemical (IHC) staining and was defined as positive if the score was 3. Fluorescent in situ hybridization was selectively performed when the IHC staining score was 2 with the cutoff value proposed in the ASCO/CAP 2013 guidelines [9].

All records were coded by an independent data monitoring body and maintained by a neutral person who was blinded to the study analysis. Survival analysis was based on 2 databases— one from Wonju Severance Hospital and the other from the Korean National Cancer Registry.

Inclusion and exclusion
Patients were eligible for inclusion if they had stage I–III TNBC and had completed planned systemic and local treatments. Patients with incomplete data; known stage IV disease; a history of previous treatment for contralateral breast cancer; systemic autoimmune disease such as systemic lupus erythematosus or scleroderma; and any other conditions that can influence the NLR such as end-stage renal disease, bone marrow dysfunction, or consumption of corticosteroids and pregnancy-related breast cancer were excluded [10-14].

Systemic treatment
Standard regimens recommended by the Health Insurance Review and Assessment Service (HIRA), a review body for the government-run health insurance system, were used.

Specifically, patient triage was mainly based on nodal metastases, which determined the major difference in regimen and the decision regarding whether taxanes should be included in the regimen. For node-negative patients, six cycles of 600 mg/m^2 fluorouracil, 60 mg/m^2 doxorubicin, and 600 mg/m^2 cyclophosphamide were intravenously administered once every 3 weeks. For patients with pathologically proven nodal metastasis, a taxane-based regimen was introduced. It consisted of four cycles of 60 mg/m^2 intravenous doxorubicin and 600 mg/m^2 intravenous cyclophosphamide repeated every 3 weeks, followed by four cycles of 175 mg/m^2 intravenous paclitaxel every 3 weeks. If patients were triaged into the preoperative chemotherapy group, the planned regimen was administered consecutively before surgery and pathological complete remission (pCR) was defined as no invasive or in situ disease in the breast and axilla.

Data collection: clinicopathological, laboratory, and survival data
Data regarding the subjects' medical history, age, pathologic results (tumor size, lymph node status), and laboratory data (including complete blood cell [CBC] count and differential white blood cell count) were collected. Data on CBC counts at baseline and follow-up were collected serially before any systemic treatment and 6 ± 1, 12 ± 1, and 24 ± 1 months after treatment completion. Patients who experienced any event of interest related to distant disease-free survival (DDFS) were censored at the respective dates of events, and follow-up serological data were not included in further analysis. NLR was calculated as the absolute neutrophil count divided by the absolute serum lymphocyte count. The cutoff values categorizing patients into the high and low NLR groups were defined using Youden's index extracted from the receiver operating characteristic (ROC) curve and the value of the area under the curve (AUC).

Statistical analysis
The baseline characteristics were summarized using descriptive statistics. For the comparison of two groups, the chi-squared test or Fisher's exact test was used for applicable categorical values, and the independent t-test was used for continuous values. NLR was analyzed for both continuous and binary scales. A linear mixed-effect model was used to determine whether the changes in the NLRs were time dependent. For the binary scale, ROC curves were generated to obtain the AUC value, and Youden's index was used to determine the appropriate cutoff value. The AUC values were also used to compare the NLRs at different time points as the most feasible prognostic factors to distinguish between high-risk and low-risk groups in terms of DDFS, distant relapse-free survival (DRFS), distant recurrence-free interval (DRFI), and overall survival (OS). Standardized definitions for efficacy end points in adjuvant breast cancer trials (the STEEP criteria) were used. The definitions of survival, DDFS, DRFS, DRFI, and OS are summarized in **Supplementary Table 1** [15]. Kaplan-Meier curves were generated to estimate the DDFS, DRFS, and OS. The log-rank test was used to compare the survival between the groups. A Cox proportional hazards model was used to estimate the hazard ratios with 95% confidence intervals for the multivariate approach. All statistical analyses were performed using IBM SPSS Statistics (version 25, IBM Corp., Armonk, USA). All *p*-values were 2-sided, and the statistically significant level was set at *p* < 0.05.

Ethical approval
This study was reviewed and approved by the Institutional Review Board of Wonju Severance Hospital (YWMR-201181) and conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived owing to the retrospective nature of the study.

RESULTS

Demographic information and clinical course of patients

In total, 210 patients were eligible for inclusion in this study. The median patient age was 50.5 years (51.5 ± 11.9 years, range: 25–83 years). Most patients presented with T1 (88/210) or T2 (97/210) tumors, and 3-quarters of the patients (149/210) did not have nodal metastasis. Preoperative systemic treatment was administered in 45 patients. Among them, 14 achieved pCR. Detailed patient demographics are shown in **Table 1**.

The median follow-up duration was 79.8 months, and 43 (20.5%) patients experienced DDFS-related events. The median duration between the initial diagnosis and DDFS-related events was 25 months (47 ± 51 months, range: 6–210 months). Most breast cancer-related events (27/29) occurred within 50 months of the initial diagnosis, with a median duration of 19 months (24.2 ± 18.8 months, range: 8–86 months). In contrast, the median duration between the initial diagnosis and non-breast second malignancy and/or death (n = 14) was 63 months (68.2 ± 55.9 months, range: 6–210 months), and half of the events (7/14) occurred 5 years after the initial diagnosis. Each case is reported in detail in **Supplementary Table 2**. Further, 30 patients died due to breast cancer (n = 24) and reasons other than breast cancer and/or unknown disease (n = 6). Locoregional or contralateral recurrence events involving only the breast, which the STEEP criteria do not include as distant disease events, occurred in nine patients, with a median duration of 39 months (69 ± 58.3 months, range: 19–173 months). Patients who eventually experienced distant relapse (n = 4) after locoregional or contralateral breast recurrence were censored at the respective dates of distant recurrence-related events.

NLRs at baseline and each follow-up time point

Data on serial NLRs were collected at the different follow-up time points. The number of patients included at each time point is shown in **Supplementary Figure 1**. The NLRs at baseline and at 6, 12, and 24 months after treatment completion were 2.24 ± 1.18 and 2.07 ± 0.93, 2.59 ± 0.98, and 1.92 ± 1.19, respectively (**Figure 1A**). Time series plots were also

Table 1. Clinicopathological characteristics of the patients

Variables	Subcategory	Values
Age	Mean ± SD	51.5 ± 11.93
	Min−max	25−83
Tumor stage	pT1	88 (41.9)
	pT2	97 (46.2)
	pT3	10 (4.8)
	pCR	14 (6.7)
	Unknown	1 (0.5)
Nodal stage	pN0	149 (71.0)
	pN1	28 (13.3)
	pN2	15 (7.1)
	pN3	12 (5.7)
	Unknown	6 (2.9)
Preop CTx	No	164 (78.1)
	Yes	45 (21.4)
	CR	14 (6.7)
	Non-CR	31 (14.8)
	Unknown	1 (0.5)
Total		210 (100.0)

Values are presented as number (%) not otherwise specified.
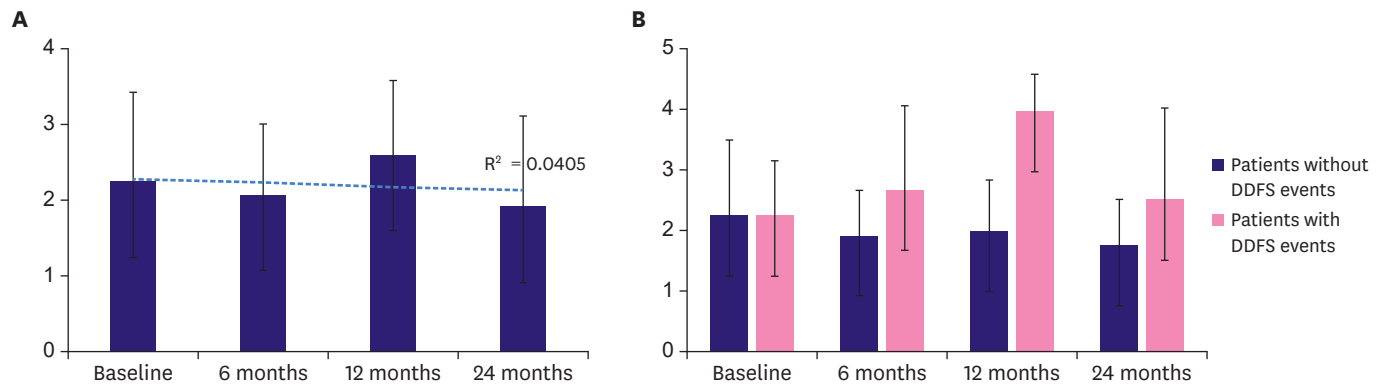SD = standard deviation; preop. CTx = preoperative chemotherapy; CR = complete remission.

Figure 1. Values of NLRs at each follow-up time points.
(A) Means with standard deviations of the NLRs at each follow-up time point are shown in the bar graph. Each purple bar represents the mean of the NLRs at baseline and 6, 12, and 24 months after completion of treatment. Each black vertical line represents the standard deviation of each mean value. The R^2 value is 0.0405, suggesting that there is no correlation or influence between time and NLR values. (B) Time series plot of the NLRs at each follow-up time point after the patients were dichotomized into two groups (with and without DDFS events) showing that patients with DDFS events had significantly higher NLRs at 6 months, 12 months, and 24 months after treatment completion, whereas the NLR values were not significantly different between the groups at baseline. P values at baseline and 6, 12, and 24 months after completion of treatment were 0.249 and < 0.001, 0.001, and 0.005, respectively.
NLR = neutrophil-to-lymphocyte ratio; DDFS = distant disease-free survival.

generated to observe the trends of the NLR (median with interquartile) at each follow-up time point, revealing an R^2 value of 0.0405 (**Figure 1A**). The linear mixed-effect model showed that changes in the NLR had no interaction with time ($p = 0.866$). However, the NLRs of patients with and without DDFS events were significantly different, with significantly higher values in patients with DDFS events, except for NLRs at baseline (**Figure 1B**).

Performance of the NLR as a prognostic factor

The performance of NLR as a prognostic factor was assessed at baseline and at 6, 12, and 24 months after treatment completion using ROC curves and AUC values. The NLRs at 6 months after treatment completion showed the strongest prognostic power (AUC = 0.745). The AUCs of the NLRs at baseline and 12 and 24 months after treatment completion were 0.593 and 0.631 and 0.693, respectively (**Supplementary Figure 2**). Cutoff values for dichotomizing patients into high-risk and low-risk groups were determined using Youden's index, and the exact values at baseline and 6 months, 12 months, and 24 months after treatment completion were 1.66 and 2.47, 2.12, and 2.50, respectively (**Supplementary Table 3**). A cutoff value of 2.47 (with the largest AUC value) was used for further analysis. Patients classified into two groups (based on NLRs > 2.47 vs. < 2.47) showed differences only in metastatic disease in the axillary lymph nodes (**Table 2**). Kaplan-Meier curves were generated, and log-rank tests were used to compare survival, DDFS, DRFS, DRFI, and OS (**Figure 2A**, **Supplementary Figures 3**-**6**). Although the Kaplan-Meier curves of the NLRs at 6 months, 12 months, and 24 months after treatment completion showed statistically significant differences in terms of survival, the NLRs at 6 months after treatment completion showed the strongest statistical power. In contrast, the initial NLRs showed statistical significance only in terms of DDFS.

Risk factors contributing to a worse prognosis

The Cox proportional hazards model showed that the presence of axillary lymph node metastases and the NLR were significant independent risk factors (**Figure 2B**). Different regimens were not assessed because they were heavily influenced by clinical staging at baseline under the HIRA coverage.
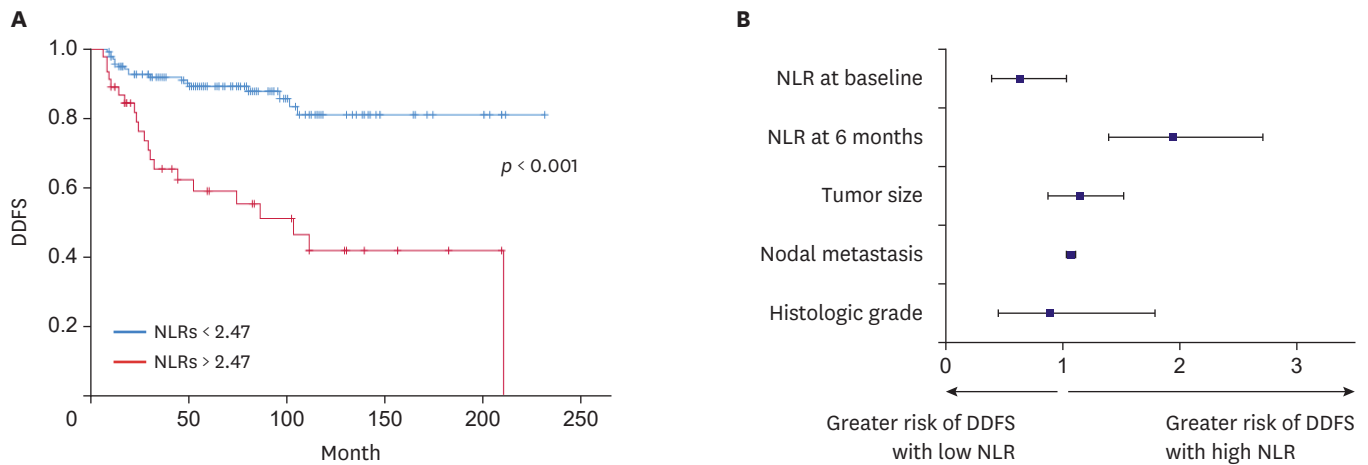
Figure 2. Survival difference between high and low NLRs at 6 months after treatment and contributing factors.
(A) The Kaplan-Meier curve generated to compare the difference in survival between high and low NLRs at 6 months after treatment completion shows statistically significant difference in DDFS (p < 0.001). (B) Risk factors predicting DDFS. Hazard ratios are estimated using the Cox proportional hazards model. The NLR at 6 months after treatment completion and nodal status are the only independent risk factors.
NLR = neutrophil-to-lymphocyte ratio; NLR_6M = NLR at 6 months after treatment completion; DDFS = distant disease-free survival.

Table 2. Patients dichotomized into 2 risk groups (NLR > 2.47 vs. < 2.47 at 6 months after completion) (n = 191)

Variables	Subcategory	Patients NLRs < 2.47	Patients NLRs > 2.47	p-value
Patients No.		145	46	
Age		51.6 ± 11.6	50.0 ± 13.0	0.424
Tumor stage	pT1	62 (42.8)	18 (39.1)	0.503
	pT2	66 (45.5)	22 (47.8)	
	pT3	7 (4.8)	2 (4.3)	
	pCR	11 (7.6)	3 (6.5)	
	Unknown	0 (0)	1 (2.2)	
Nodal involvement	Absence	114 (78.6)	25 (54.3)	0.002
	Presence	28 (19.3)	19 (41.3)	
	Unknown	3 (2.1)	2 (4.3)	
Preop. CTx	No	111 (77.1)	35 (76.1)	0.889
	Yes	33 (22.9)	11 (23.90)	
	pCR*	9 (27.3)	3 (27.2)	
	Non-pCR*	24 (72.7)	8 (72.7)	
	Unknown	1 (0.7)	0 (0)	
NLRs	Baseline	2.12 ± 1.17	2.59 ± 1.18	0.021
	6m after	1.66 ± 0.41	3.37 ± 0.94	< 0.001
	12m after	2.20 ± 3.20	3.33 ± 2.46	0.047
	24m after	1.71 ± 0.69	2.80 ± 2.03	< 0.001

Values are expressed as mean ± standard deviation or number (%).
NLR = neutrophil-to-lymphocyte ratio; preop. CTx = preoperative chemotherapy; pCR = pathological complete remission.
*Number within patients with preoperative chemotherapy.

DISCUSSION

The present study demonstrated that the NLR at 6 months after treatment completion can be a useful prognostic factor in patients with TNBC and can serve as a potential biomarker to escalate treatment after completion of the standard treatment.

Improvements in cancer genomics have led researchers to understand the intrinsic subtypes of breast cancer, and clinicians soon realized that patients with each subtype show totally different clinical behaviors [16-19]. Patients with TNBC were four times more likely to

experience visceral metastasis within 5 years of diagnosis than those with all other subtypes, even though the treatment duration for TNBC is shorter than that for other subtypes. Patients with ER-positive breast cancer are exposed to 5–10 years of hormonal treatment, and patients with HER2-positive disease are exposed to 1 year of HER2-targeted therapy [3-5]. However, appropriate treatment escalation immediately after completion of standard treatment is urgent as the risk of distant recurrence in patients with TNBC peaks at approximately 3 years after diagnosis, with a rapid decline thereafter [7,14,19,20]. Our data also showed that most breast cancer-related DDFS events (27/29) occurred within 50 months of the initial diagnosis, which is consistent with the findings of previous studies and suggests a pertinent strategy to identify high-risk patients who could benefit from appropriate escalation of adjuvant treatment [1,2,7].

We compared NLRs at various time points, including baseline and common follow-up intervals of 6, 12, and 24 months to determine the follow-up time point at which the NLR can become the most powerful prognostic biomarker. First, we confirmed that the NLRs at each time point were independent of elapsed time based on the R^2 from time series plots and the linear mixed-effect model ($p = 0.086$). We then compared NLRs at each time point because although the NLR is related to patient prognosis [10-12], most previous studies have only investigated one or two follow-up time points, and it is unclear whether the baseline NLR or the NLRs at certain follow-up time points have the strongest and the most relevant prognostic power [14,21-23].

The NLRs calculated after the completion of standard treatment are important because they can reflect the altered immune status after chemotherapy. Two recent randomized clinical trials using immunotherapy in patients with early TNBC reported that the status of programmed death-ligand 1 did not affect the efficacy of immune checkpoint inhibitors, suggesting that precedent profiling of the immunologic status of patients with early TNBC before treatment might not be pertinent to disease prognosis [1,2]. This finding was also supported by profiling data of paired primary and recurrent TNBC tumors, which revealed that molecular evolution of TNBC through chemotherapy selection pressure mostly influenced immune activity-associated gene expression signatures [1,2,24]. However, baseline NLRs have shown diverse features with controversial findings in terms of their influence on the survival of patients with TNBC. Although some studies have reported a positive correlation between the baseline NLR and patients' survival (AUC value not reported), recent studies have suggested the possibility of a factor that has stronger prognostic power than the baseline NLR after it failed to show statistically significant performance as a biomarker to predict patient survival [12,14,24,25].

In the present study, we confirmed that the NLR at 6 months after treatment completion could be the most relevant biomarker in patients with TNBC among diverse follow-up time points, whereas the baseline NLR showed only the survival trend. Although the NLRs at 12 and 24 months after treatment completion showed poor performance, the NLR at 6 months after treatment completion showed the largest AUC value (0.745); therefore, we considered it as the best marker. Moreover, each analysis included patients without evidence of DDFS events at that time point after patients with DDFS events were sorted according to the time of events. This indicates that the NLR can be a promising factor if each test point is considered the starting point. However, the beginning of the follow-up is the most important time point when a useful biomarker would help identify high-risk patients who need escalation.

Multivariate analysis revealed that lymph node metastasis and the NLR at 6 months after treatment completion were independent prognostic factors [10-12]. Conventional risk factors such as lymph node status do not always predict prognosis concisely and require complementary factors to improve its capacity to predict prognosis [26]. Patients with TNBC have a lower chance of nodal metastasis than those with other subtypes, but they have a worse prognosis even after adjusting for tumor size and nodal stage [27].

A high NLR and its influence on patient survival might be the result of pro-tumor activities of neutrophils and suppressed lymphocyte function [1,2,28,29]. Neutrophils are the first responders to acute inflammation. Rather than simply killing the microorganisms, they are involved in more complicated mechanisms, performing a pivotal role in chronic inflammatory diseases such as cancer. Neutrophils can play pro-tumor roles through multiple mechanisms, such as releasing reactive oxygen/reactive nitrogen species, neutrophil-released enzymes, and neutrophil extracellular traps and self-education with their own cytokines and chemokines [28]. However, there is a caveat in the role of neutrophils as the positive and negative impacts of neutrophils on survival have been reported. However, a lack of lymphocytes could lead to disease recurrence due to adverse effects on immune surveillance [29]. Moreover, they may be inhibited by several regulatory systems, suggesting an approach to increase not only the number but also the functional aspects of lymphocytes [1,2,29].

The limitations of this study are mainly due to its retrospective nature. More than 90% patients had at least three NLR values. However, missing data regarding blood tests may lead to selection bias [14]. Since each NLR at a certain time point produced significant predictions as a prognostic factor, a structured study regarding NLRs can help completely understand the dynamics and mechanisms behind increased NLR and tumor evolution. In conclusion, NLRs after the completion of standard treatment were significantly associated with DFS and OS in patients with TNBC. Although the baseline NLR could show only trend in survival, the NLR at 6 months after treatment completion was the most feasible prognostic factor. Substantial efforts in both clinical and translational research are required to elucidate the mechanisms underlying the clinical features.

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIALS

Supplementary Table 1
Events included in the OS, DDFS, DRFS, and DRFI [15]

Click here to view

Supplementary Table 2
Patients with events related to DDFS

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Supplementary Table 3
Coordinates of the receiver operating characteristic curve of NLRs at each follow-up point: baseline, 6, 12, and 24 months after treatment completion

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Supplementary Table 4
HRs and CIs of the variables*

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Supplementary Figure 1
The number of patients included at each point of the analysis. Altogether, 210 patients were enrolled at the baseline, and 191, 171, and 160 patients were included at 6, 12, and 24 months, respectively, after treatment completion.

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Supplementary Figure 2
ROC curves and AUC of NLR after the completion of treatment among patients with TNBC, excluding patients receiving neoadjuvant treatment. The ROC curves and AUCs show that the NLR at 6 months after treatment completion showed the best performance as a binary classifier based on the distant disease-free survival of patients with TNBC.

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Supplementary Figure 3
Kaplan-Meier curves were generated to analyze the difference in survival between high and low NLRs at (A) baseline, (B) 6 months, (C) 12 months, and (D) 24 months after treatment completion. Different cut-off values were applied for each follow-up point according to Youden's index (described in **Supplementary Table 3**). All graphs show statistically significant differences in the DDFS.

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Supplementary Figure 4
Kaplan-Meier curves were generated to analyze the difference in survival between high and low NLRs at (A) baseline, (B) 6 months, (C) 12 months, and (D) 24 months after treatment completion. Different cut-off values were applied for each follow-up point according to Youden's index (described in **Supplementary Table 3**). Each graph shows statistically significant differences in DRFS, except the baseline NLRs ($p = 0.670$).

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Supplementary Figure 5
Kaplan-Meier curves were generated to analyze the difference in survival between high and low NLRs at (A) baseline, (B) 6 months, (C) 12 months, and (D) 24 months after treatment completion. Different cut-off values were applied for each follow-up point according to

Youden's index (described in **Supplementary Table 3**). Each graph shows statistically significant differences in the DRFI, except the baseline NLRs ($p = 0.103$).

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Supplementary Figure 6
Kaplan-Meier curves were generated to analyze the difference in survival between high and low NLRs at (A) baseline, (B) 6 months, (C) 12 months, and (D) 24 months after treatment completion. Different cut-off values were applied for each follow-up point according to Youden's index (described in **Supplementary Table 3**). Each graph shows statistically significant differences in the OS, except the baseline NLRs ($p = 0.227$).

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