

Prognostic Significance of Neutrophil-to-lymphocyte Ratio in Luminal Breast Cancers With Low Levels of Tumour-infiltrating Lymphocytes

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Abstract. *Background/Aim:* This study aimed to improve the prognostic significance of neutrophil-to-lymphocyte ratio (NLR) and tumour-infiltrating lymphocytes (TILs). *Patients and Methods:* In this retrospective study, NLR and TIL data from 677 operated breast cancer patients were analysed. The cut-off value of NLR was set at 2.72, and TIL levels were classified as low (<10%), intermediate (≥10 to <50%), and high (≥50%). *Results:* Recurrence-free survival (RFS) was significantly longer in patients with low NLR (n=459) than in those with high NLR (n=218) (p=0.0383). In ER-positive/HER2-negative and TIL-low breast cancers, there were significant associations between NLR levels and RFS (p=0.0129) or overall survival (OS) (p=0.0046). On multivariate analysis, NLR was a significant and independent factor for OS (hazard ratio=3.78; 95% confidence interval=1.21-14.17; p=0.022). *Conclusion:* These data may be useful for predicting patient prognosis and understanding the clinical significance of immune status in breast cancers.

The prognostic significance of the neutrophil-to-lymphocyte ratio (NLR) for breast cancer has been previously demonstrated. A meta-analysis of 39 studies including 17,079 patients showed that elevated NLR was significantly associated with poor overall survival (OS) [hazard ratio

(HR)=1.78; 95% confidence interval (CI)=1.49-2.13; p<0.001] and poor disease-free survival (DFS) (HR=1.60; 95%CI=1.42-1.96; p<0.001) (1). Noh *et al.* have reported that although high NLR (≥2.5) was a significant predictor of a lower disease-specific survival rate of 442 breast cancer patients, luminal A subtype (oestrogen receptor (ER)-positive and/or progesterone receptor (PgR)-positive and human epidermal growth factor receptor 2 (HER2)-negative) was the only subtype in which the relationship was consistent (87.7% vs. 96.7%; p=0.009) (2). In addition, in ER/PgR-positive and HER2-negative breast cancers treated with neoadjuvant therapy, high NLR (>2.25) correlated with poorer recurrence-free survival (RFS) and OS (p=0.001 and p<0.001, respectively) (3). In contrast, another meta-analysis has reported that the association between high NLR and inferior prognosis was greater for ER-negative and HER2-negative (triple-negative; TN) breast cancer (4). Thus, although the prognostic significance of NLR for operated breast cancers has been confirmed, its significance as a prognostic factor for each breast cancer subtype remains unknown.

Many studies have focused on the role of tumour-infiltrating lymphocytes (TILs) in breast cancer as both a prognostic and predictive factor. In an analysis of samples recruited for phase III adjuvant trial in lymph node metastasis-positive breast cancers comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy (BIG 02-98), the DFS of patients with lymphocyte-predominant breast cancer (LPBC) was significantly associated with the TN subtype (p=0.014) but not in the ER-positive and HER2-negative subtype (p=0.746) (5). Consistent with this report, there was no significant association between TIL levels and DFS or OS in a meta-analysis of 25 studies comprising a total of 22,964 patients (6). However, TILs were significantly associated with improved DFS (HR=0.82; 95%CI=0.76-0.88) and OS (HR=0.79; 95%CI=0.71-0.87) in patients with the TN subtype.

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Interestingly, high levels of TILs were a predictor for longer OS in the TN subtype (HR=0.92; 95%CI=0.86-0.99; $p=0.032$) but increased TIL levels were associated with shorter OS in luminal (hormone receptor positive) and HER2-negative tumours (HR=1.10; 95%CI=1.02-1.19; $p=0.011$) in the pooled analysis of patients treated with neoadjuvant therapy (7). In addition, high TIL distributions were significantly associated with shorter survival time from recurrence in ER-positive and HER2-negative breast cancers (8, 9).

Thus, although the prognostic significance of NLR and TILs has been established, its utility seems to be dependent on subtypes. The increase in TIL levels is significantly associated with pathological complete response (pCR) to chemotherapy irrespective of breast cancer subtypes (7, 10). In contrast, the association between low levels of NLR and higher frequencies of pCR seems to be restricted to the TN subtype (11, 12).

Both NLR and TILs are recognised as immunological factors in systemic host immunity and local anti-cancer immunity, respectively, and the mechanisms involved in the processes related to prognosis may be different. However, this research area is yet to be fully explored. In the present study, we evaluated the associations between NLR and TIL levels considering prognosis in operated breast cancers retrospectively, especially focusing on subtypes in order to identify subgroups in which NLR and TILs can best predict prognosis.

Patients and Methods

Patient eligibility. A total of 1081 breast cancer patients who underwent surgery between October 2008 and March 2017 at the Hyogo College of Medicine were screened for inclusion in this retrospective study. Of these, patients with non-invasive carcinoma (n=206), stage IV (n=19), ipsilateral (n=7) breast cancers, and patients with missing NLR data (n=38), TILs (n=125), and clinical data (n=9) were excluded. The remaining 677 patients with histopathologically diagnosed breast cancer were included in the present study. RFS was defined from the time of operation to the first event, including invasive ipsilateral breast cancer, local recurrence, distant recurrence, and death due to any reason. OS was defined from the time of operation to death due to any reason. During the median follow-up of 36.5 months (range=0.7-107.9 months), events of RFS and OS occurred in 72 and 29 cases, respectively. This study was approved by the ethics committee of the Hyogo College of Medicine (No. 1886) in accordance with the Declaration of Helsinki. The institute's ethics committee did not require written informed consent from patients since this was a retrospective observational study.

Subtypes classification. Samples were classified as ER-positive if 1% or more cancer cells showed positive nuclear staining. Samples were classified as HER2-positive if they were found to have an immunohistochemical score (IHC) of 3 on membrane staining; in cases with an IHC score of 2, fluorescence *in situ* hybridisation was performed following the criteria of ASCO CAP HER2 testing (13).

Subtypes were divided into ER-positive/HER2-negative (n=491), HER2-positive (n=102), and ER-negative/HER2-negative (n=84). Ki67 expression levels were determined immunohistochemically by nuclear staining and samples with staining of <25% were categorised as Ki67-low (<25%) and those with staining $\geq 25\%$ were categorised as Ki67-high samples ($\geq 25\%$).

Determination of the NLR and TIL levels. Baseline data on patient NLR levels within 4 weeks before the operation were retrieved from patient records. In those treated with preoperative chemotherapy (n=169), data were obtained prior to preoperative chemotherapy. NLR was defined as neutrophil counts divided by lymphocyte counts and neutrophil and lymphocyte counts were measured automatically using Sysmex XN-9000 or XN-1000 haematological analysers (Sysmex Corporation, Kobe, Japan) as described previously (14). The cut-off value of NLR for RFS was previously defined as 2.72, according to the receiver operating characteristic curve (14).

TIL levels were determined in haematoxylin and eosin-stained samples obtained during operation or biopsy samples for patients treated with preoperative chemotherapy as described previously (15). Samples were classified as low (<10%; n=458), intermediate (≥ 10 to <50%; n=160), and high ($\geq 50\%$; n=59) based on their TIL levels.

Statistical analysis. The relationship between clinicopathological characteristics and NLR or TIL levels were calculated using Fisher's exact test. RFS and OS between different groups were compared by Kaplan-Meier plots, and statistical significance was calculated using the log-rank test. Unadjusted HRs and 95% CIs for RFS in each subgroup were calculated using a Cox proportional-hazards model. Univariate and multivariate analyses in the ER-positive/HER2-negative and TIL-low group for RFS or OS were determined using the Cox proportional-hazards model. The NLR levels were compared to TIL categories in each subtype using the Kruskal-Wallis test. All statistical analyses were performed using JMP® Pro Version 13 (SAS Institute Inc., Cary, NC, USA), and statistical significance was set at $p<0.05$.

Results

Associations between clinicopathological characteristics and NLR or TIL levels. The characteristics of the 677 patients enrolled in this study were compared according to their NLR or TIL levels (Table I). Tumour grade 1 (71.6%) and Ki67-low (70.5%) cancers were significantly more frequent in the NLR-low group than in the tumour grade 2+3 (63.5%, $p=0.035$) and Ki67-high (62.4%, $p=0.036$) groups, respectively. In contrast, there were significant associations between TIL levels and tumour grade ($p<0.0001$), ER positivity ($p<0.0001$), PgR positivity ($p<0.0001$), HER2 status ($p=0.0007$), subtype ($p<0.0001$), Ki67 expression level ($p<0.0001$), and chemotherapy administration ($p<0.0001$).

Prognosis of patients according to NLR or TIL levels and associations between NLR and TIL levels. The RFS of NLR-low patients was significantly longer than that of NLR-high patients ($p=0.0383$) (Figure 1A). Similarly, OS was

Table I. Clinicopathological characteristics of breast cancers according to neutrophil-to-lymphocyte ratio (NLR) or tumour-infiltrating lymphocyte (TIL) levels.

	NLR-low ^a (n=459)	High (n=218)	p-Value	TIL-low ^b (n=458)	Intermediate (n=160)	High (n=59)	p-Value
Menopausal status ^c							
Pre-	152 (63.1) ^d	89 (36.9)	0.058	158 (65.6)	58 (24.1)	25 (10.4)	0.500
Post-	303 (70.5)	127 (29.5)		296 (68.8)	100 (23.3)	34 (7.9)	
Tumour size							
≤2 cm	276 (70.4)	116 (29.6)	0.096	276 (70.4)	86 (21.9)	30 (7.7)	0.182
>2 cm	183 (64.2)	102 (35.8)		182 (63.9)	74 (26.0)	29 (10.2)	
Lymph node metastasis ^e							
Negative	313 (69.4)	138 (30.6)	0.189	314 (69.6)	99 (22.0)	38 (26.7)	0.311
Positive	142 (64.3)	79 (35.7)		141 (63.8)	59 (26.7)	21 (9.5)	
Tumour grade ^f							
1	280 (71.6)	111 (28.4)	0.035	312 (79.8)	64 (16.4)	15 (3.8)	<0.0001
2+3	153 (63.5)	88 (36.5)		112 (46.5)	85 (35.3)	44 (18.3)	
Oestrogen receptor							
Positive	377 (68.2)	176 (31.8)	0.671	408 (73.8)	117 (21.2)	28 (5.1)	<0.0001
Negative	82 (66.1)	42 (33.9)		50 (40.3)	43 (34.7)	31 (25.0)	
Progesterone receptor ^g							
Positive	291 (68.2)	136 (31.9)	0.784	322 (75.4)	86 (20.1)	19 (4.5)	<0.0001
Negative	134 (67.0)	66 (33.0)		103 (51.5)	60 (30.0)	37 (18.5)	
HER2 status							
Negative	390 (37.8)	185 (32.2)	0.999	405 (70.4)	121 (21.0)	49 (8.5)	0.0007
Positive	69 (67.6)	33 (32.4)		53 (52.0)	39 (38.2)	10 (9.8)	
Subtypes							
ER+/HER2-	336 (68.4)	155 (31.6)	0.738	372 (75.8)	96 (19.6)	23 (4.7)	<0.0001
HER2+	69 (67.6)	33 (32.4)		53 (52.0)	39 (38.2)	10 (9.8)	
ER-/HER2-	54 (64.3)	30 (35.7)		33 (39.3)	25 (29.8)	26 (31.0)	
Ki67 expression level ^{h,i}							
Low	313 (70.5)	131 (29.5)	0.036	351 (79.1)	79 (17.8)	14 (3.2)	<0.0001
High	141 (62.4)	85 (37.6)		100 (44.2)	81 (35.8)	45 (19.9)	
Chemotherapy ^j							
No	333 (68.5)	153 (31.5)	0.522	359 (73.9)	89 (18.3)	38 (7.8)	<0.000
Yes	124 (66.0)	64 (34.0)		98 (52.1)	69 (36.7)	21 (11.2)	

^ahigh: ≥2.72; low: <2.72; ^bhigh: ≥50%, intermediate: ≥10 to >50%, low: <10%; ^c6 patients were unknown; ^d(%); ^e5 patients were not examined; ^f45 patients were unknown; ^g50 patients were unknown; ^hlow: <25%, high: ≥25%; ⁱ7 patients were unknown; and ^j3 patients were unknown.

marginally longer in NLR-low patients than that in NLR-high patients, but the difference was not significant ($p=0.0617$) (Figure 1B). There was no significant association between TIL levels and RFS ($p=0.984$) or OS ($p=0.881$). NLR levels were not significantly different in the low, intermediate, and high TIL groups in the ER-positive/HER2-negative ($p=0.323$), HER2-positive ($p=0.864$), and ER-negative/HER2-negative ($p=0.288$) subtypes (Figure 2).

HRs and 95% CIs of NLR-low patients for RFS in each subgroup. Since NLR but not TIL levels were associated with RFS, the relationship between NLR levels and RFS was investigated in subgroups (Figure 3). NLR-low patients showed consistently longer RFS than NLR-high patients irrespective of menopausal status, tumour size, lymph node metastasis, nuclear grade, and chemotherapy administration

(Figure 3). In the ER-positive/HER2-negative subtype (HR=0.45; 95%CI=0.25-0.80), RFS was better in the NLR-low group. In contrast, RFS was similar between NLR-low and NLR-high groups in the HER2-positive (HR=1.05; 95%CI=0.28-3.99) and ER-negative/HER2-negative (HR=1.16, 95%CI=0.40-3.35) subtypes. NLR-low patients also showed consistently longer RFS in the Ki67-high (HR=0.51; 95%CI=0.26-0.98) and TIL-low (HR=0.56; 95%CI=0.32-0.98) groups as compared with the Ki67-low (HR=0.88; 95%CI=0.43-1.81), TIL-intermediate (HR=0.70; 95%CI=0.26-1.89) and TIL-high (HR=0.98; 95%CI=0.18-5.42) groups.

Prognosis of NLR-low and -high patients according to subtypes or TIL levels. The RFS of NLR-low patients was significantly longer than that of NLR-high patients for the

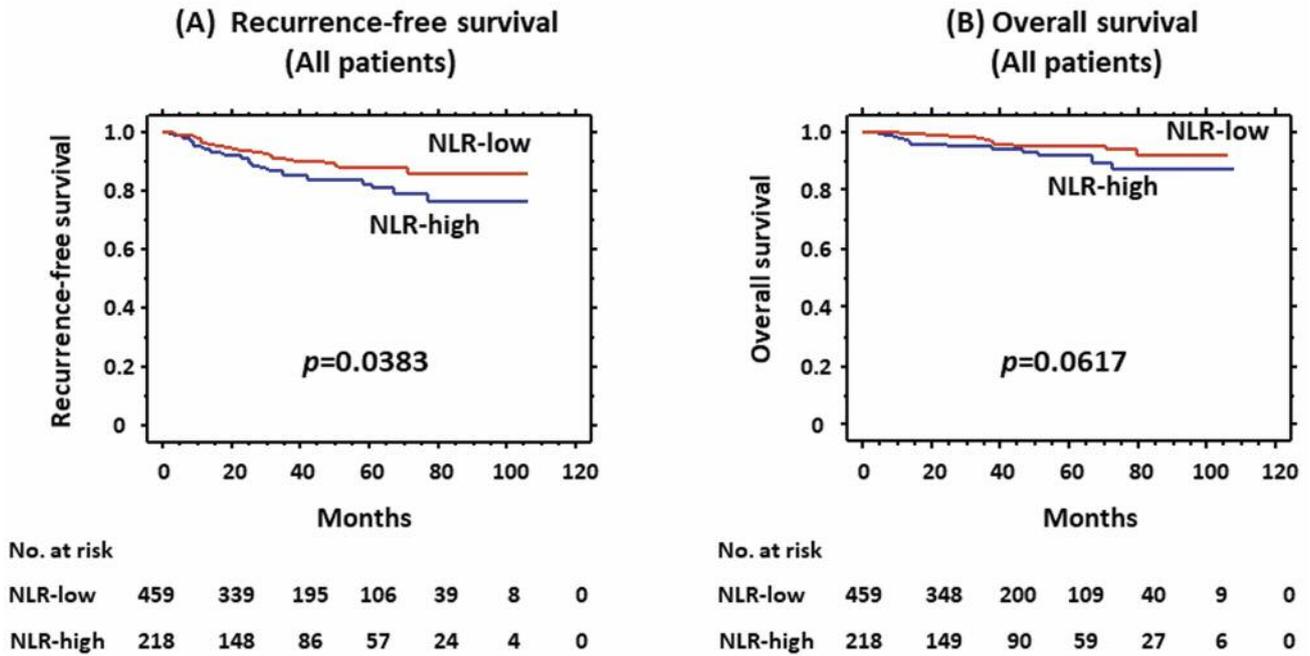


Figure 1. Recurrence-free survival (A) and overall survival (B) of all patients according to neutrophil-to-lymphocyte ratio (NLR) levels (low and high).

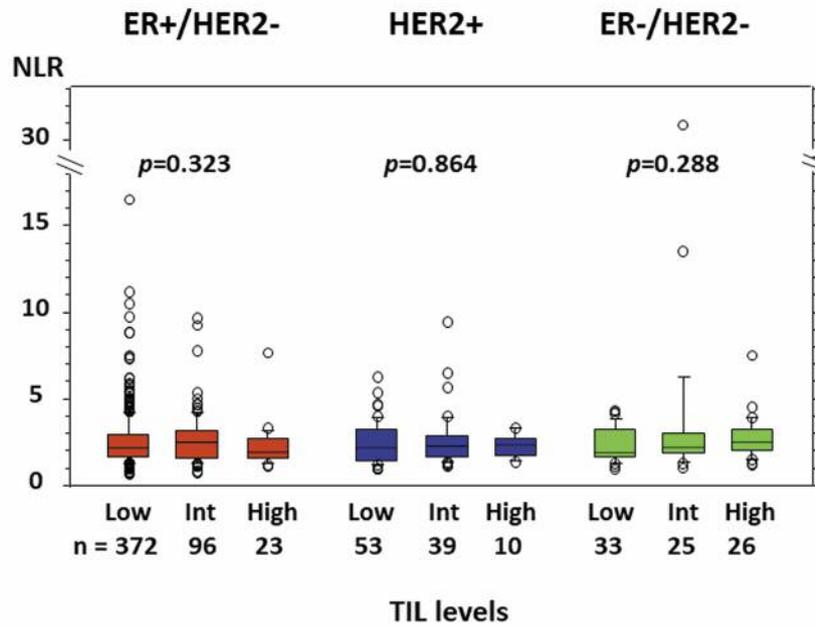


Figure 2. Relationship between neutrophil-to-lymphocyte ratio (NLR) and tumour-infiltrating lymphocyte (TIL) levels for each breast cancer subtype. ER: Oestrogen receptor; HER2: human epithelial growth factor receptor 2; TIL-high, $\geq 50\%$; TIL-intermediate, ≥ 10 to $>50\%$; TIL-low, $<10\%$.

ER-positive/HER2-negative subtype ($p=0.0056$) (Figure 4A). In contrast, there was no significant association between RFS and NLR levels in the HER2-positive ($p=0.937$) and ER-negative/HER2-negative ($p=0.771$) subtypes (Figure 4B,

C). A longer RFS was recognised in the TIL-low group ($p=0.0385$) but not in the TIL-intermediate ($p=0.478$) and TIL-high ($p=0.981$) groups (Figure 5). Since the positive relationships between NLR levels and RFS were recognised

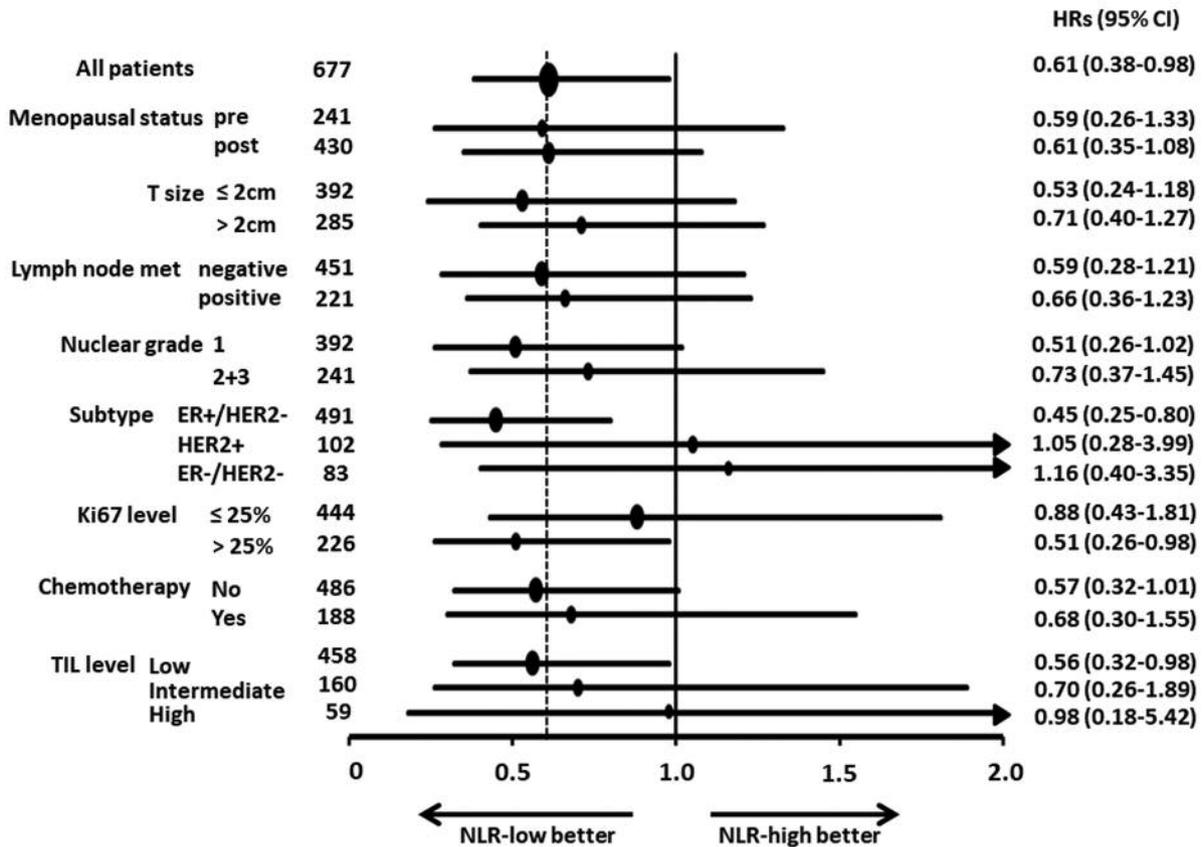


Figure 3. Forest plots of neutrophil-to-lymphocyte ratio (NLR) levels for recurrence-free survival. The dashed line shows a hazard ratio (HR) of 0.61 and a 95% confidence interval (CI) of 0.38-0.98 in all patients. ER: Oestrogen receptor; HER2: human epithelial growth factor receptor 2; TIL: tumour-infiltrating lymphocyte.

in the ER-positive/HER2-negative subtype and the TIL-low group, these categories were further combined. Significantly longer RFS ($p=0.0129$) and OS ($p=0.0046$) were found in the ER-positive/HER2-negative group with low TIL counts (Figure 6).

Univariate and multivariate analyses in the ER-positive/HER2-negative group with low TIL counts. Tumour size (HR=3.45; 95%CI=0.74-7.20), lymph node metastasis (HR=2.13; 95%CI=1.07-4.18), tumour grade (HR=2.60; 95%CI=1.23-5.25), Ki67 expression levels (HR=3.70; 95%CI=1.79-7.36), and NLR (HR=2.28; 95%CI=1.15-4.50) were significant predictive factors for RFS by univariate analysis (Table II). In multivariate analysis that included these significant factors, tumour size (HR=2.55; 95%CI=1.15-5.86; $p=0.021$) but not NLR (HR=1.78; 95%CI=0.86-3.68; $p=0.120$) was a significant factor for RFS (Table II). Multivariate analysis including Ki67 expression and NLR confirmed that NLR was an independent and significant factor for OS (HR=3.78; 95%CI=1.21-14.17; $p=0.022$) (Table III).

Discussion

In the present study, the prognostic significance of NLR for operated breast cancers was prominent in the ER-positive/HER2-negative subtype and TIL-low group. Multivariate analysis revealed that low NLR was an independent prognostic factor for OS in this subgroup. There was no significant association between NLR and TIL levels in either breast cancer subtype. Our results suggest that NLR and TILs affect the prognosis of breast cancer patients through different mechanisms but this hypothesis still needs to be confirmed in future studies. Yoon *et al.* have reported that NLR was not associated with LPBC ($p=0.099$) but the absolute neutrophil count (ANC) was significantly lower in the LPBC (TIL levels $\geq 50\%$) group compared with non-LPBC group ($p=0.023$) (16). This negative association between TILs and ANC was consistently significant in ER-negative ($p=0.0341$) but not in ER-positive breast cancers. In the study reported by Lee *et al.*, NLR was not associated with cluster of differentiation 8 (CD8)-positive T cells ($p=0.518$) or forkhead box protein 3 (FOX-P3)-positive T

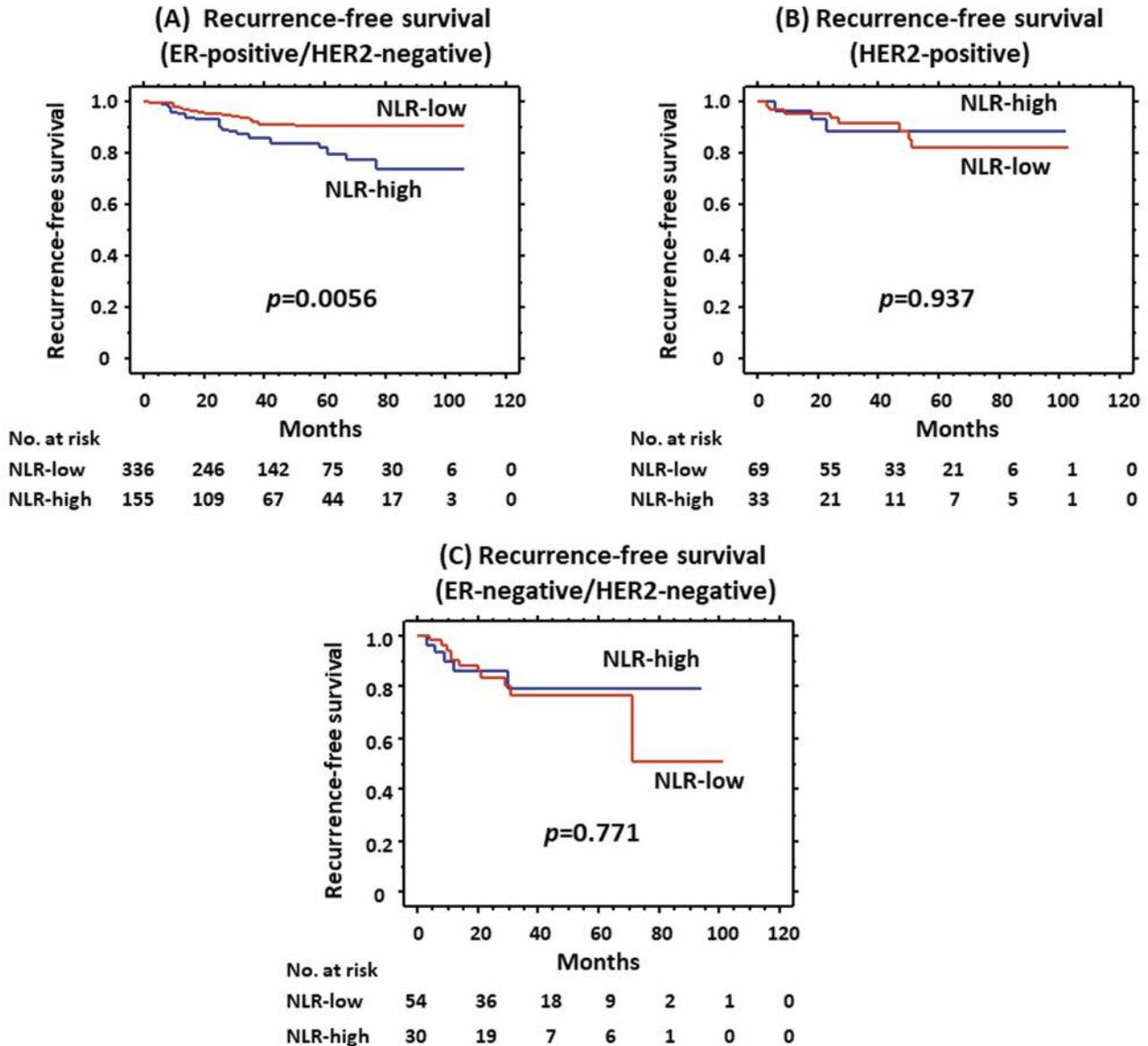


Figure 4. Recurrence-free survival of patients according to neutrophil-to-lymphocyte ratio (NLR) levels in (A) oestrogen receptor (ER)-positive/human epithelial growth factor receptor 2 (HER2)-negative, (B) HER2-positive, and (C) ER-negative/HER2-negative subtypes.

cells ($p=0.307$). In contrast, there was a significant association between absolute lymphocyte count and CD8-positive T cells ($p=0.004$) (17). Based on these inconsistent results, we speculate that NLR levels do not represent the degree of anti-cancer immunity and that TIL levels do not influence the peripheral blood marker NLR in breast cancer patients.

The prognostic value of NLR is not necessarily consistent across breast cancer subtypes. The data from our study support the results of previous studies that demonstrated a significant association between high levels of NLR and shorter prognosis in patients with ER-positive/HER2-

negative breast cancer subtype (2, 3). However, our results differ from those of a previous meta-analysis that found an association between high levels of NLR and inferior prognosis, which was greater for the TN subtype (4). This difference could potentially partly be explained by the fact that we defined the optimal cut-off of NLR at 2.72 using the ROC curve in the total population (14), whereas the meta-analysis used variable cut-off values for NLR from 1.9 to 5.0. As shown in the subgroup analysis, in addition to HER2-positive and TN subtypes, HRs were close to 1.0 in Ki67-low and TIL-high groups (Figure 3). These data are in line with the heterogeneous contribution of NLR for patient

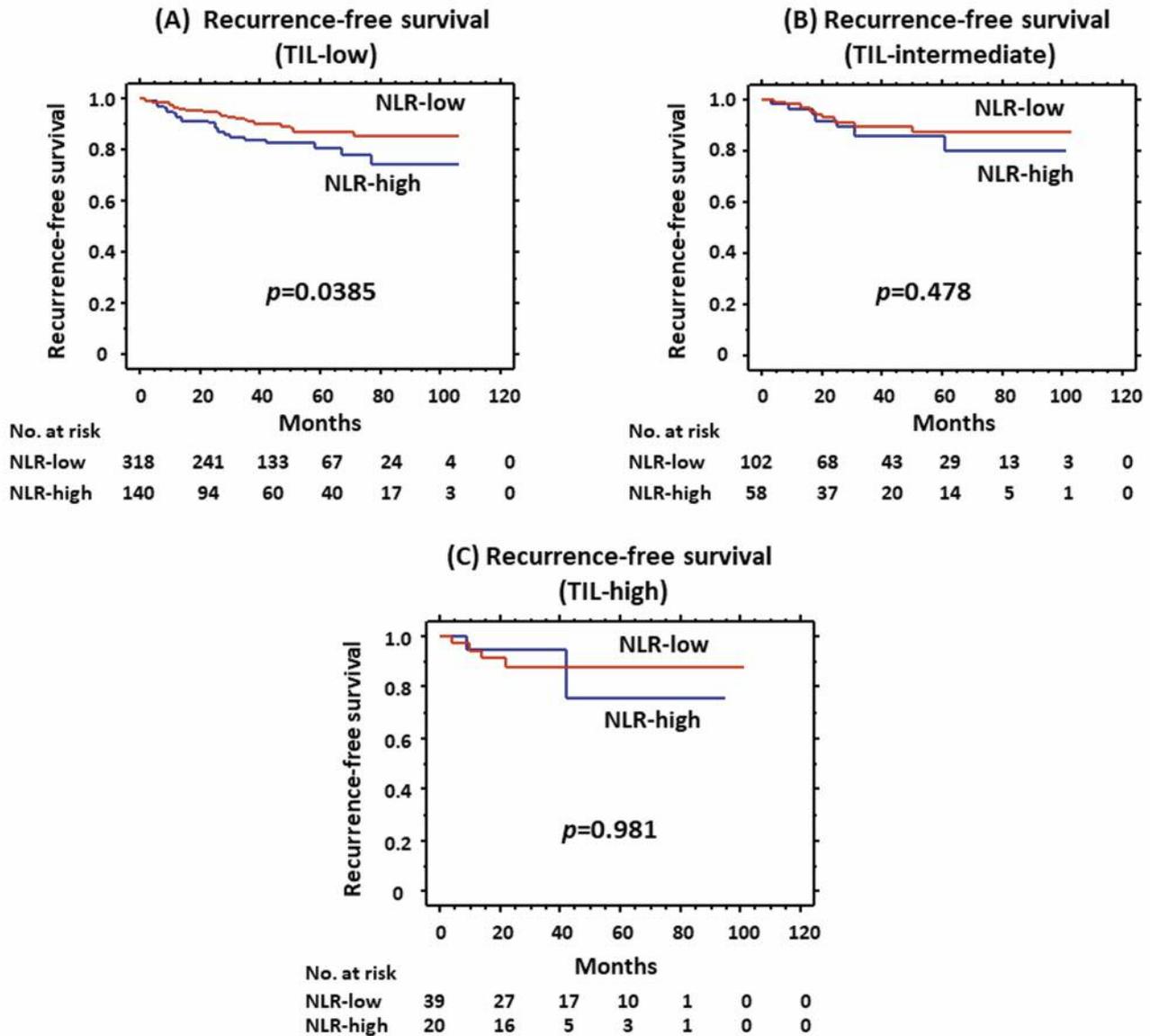


Figure 5. Recurrence-free survival of patients according to neutrophil-to-lymphocyte ratio (NLR) levels in (A) tumour-infiltrating lymphocyte (TIL)-low, (B) TIL-intermediate, and (C) TIL-high groups.

prognosis. In a previous study, we demonstrated improved prognosis in patients with low NLR is significant in those with a high absolute lymphocyte count (ALC) (14). Our results suggest that the unfavourable effects induced by neutrophils seem to affect patients with high levels of ALC; however, if ALC was low, neutrophil counts had no impact. Thus, the impact of NLR seems to be different depending on host immunity or the microenvironment of breast cancers. Although our results are not conclusive due to the small number of patients (n=59), NLR did not affect patients' prognosis in the TIL-high group (Figure 5). In an immune active microenvironment, such as high TIL count, NLR may have less influence on prognosis. In contrast, an immune

inactive microenvironment such as one with low TIL levels and high NLR is speculated to have an important role in prognosis.

According to Yoon *et al.* (16), ALC may be linked to TIL levels in the tumour. However, neutrophils have been also known to suppress immune reactions and promote tumour growth (18, 19). In patients with colorectal cancer with high blood NLR, significantly higher levels of cytokines including inflammatory cytokines, angiogenic cytokines, and epidermal growth factor ligands have been reported (20). These data highlight the usefulness of NLR, which reflects the tumour microenvironment associated with inflammatory components; thus, an increase in NLR may indicate a tumour-suppressive

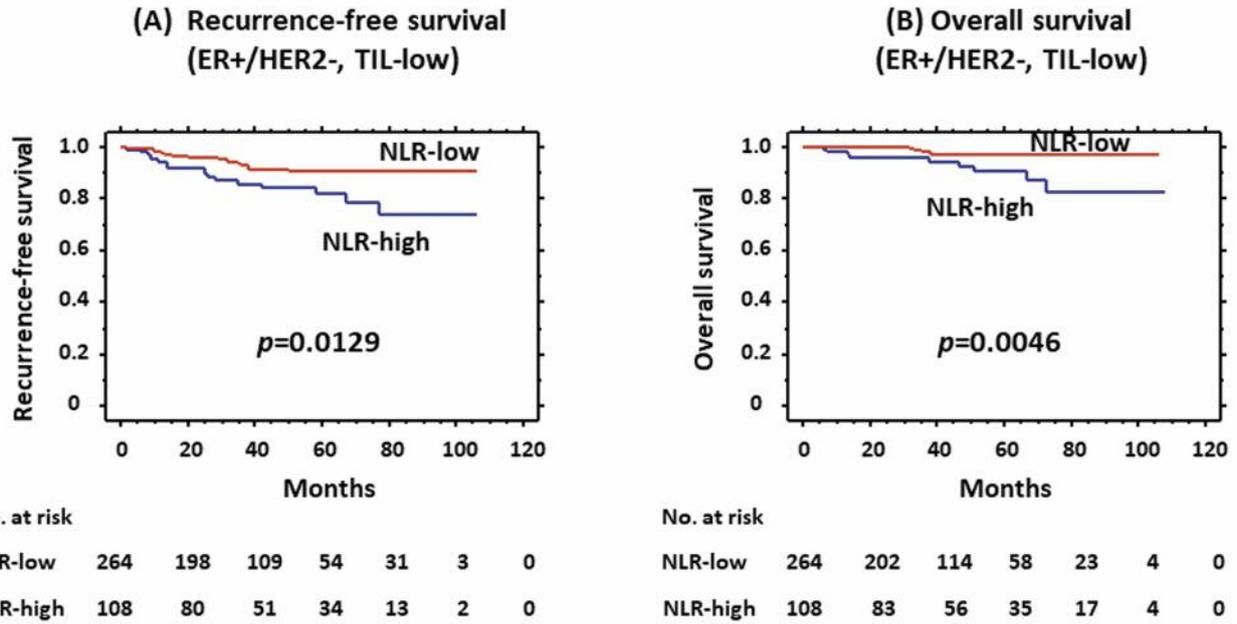


Figure 6. Recurrence-free survival (A) and overall survival (B) of patients according to neutrophil-to-lymphocyte ratio (NLR) levels in the oestrogen receptor (ER)-positive/human epithelial growth factor receptor 2 (HER2)-negative with tumour-infiltrating lymphocyte (TIL)-low group.

Table II. Univariate and multivariate analyses of recurrence-free survival in the ER-positive/HER2-negative with TIL-low group.

	n	Univariate analysis HR (95% CI) ^a	p-Value	Multivariate analysis HR (95% CI) ^a	p-Value
Menopausal status					
Pre-	135	1.00	0.513		
Post-	234	1.27 (0.63-2.70)			
Tumour size					
≤2.0 cm	234	1.00	0.0004	1.00	0.021
>2 cm	138	3.45 (1.74-7.20)		2.55 (1.15-5.86)	
Lymph node metastasis					
Negative	259	1.00	0.031	1.00	0.576
Positive	111	2.13 (1.07-4.18)		1.24 (0.58-2.65)	
Tumour grade					
1	277	1.00	0.013	1.00	0.339
2+3	71	2.60 (1.23-5.25)		1.50 (0.64-3.35)	
Ki67 expression level ^b					
Low	312	1.00	0.0006	1.00	0.067
High	58	3.70 (1.79-7.36)		2.19 (0.95-4.90)	
Chemotherapy					
No	320	1.00	0.076		
Yes	52	2.09 (0.92-4.32)			
NLR level ^c					
Low	264	1.00	0.018	1.00	0.120
High	108	2.28 (1.15-4.50)		1.78 (0.86-3.68)	

^aHazard ratio (95% confidence interval), ^blow: <25%, high: ≥25%, ^chigh: ≥2.72, low: <2.72.

immune microenvironment. In the present study, we demonstrated that the prognostic significance of NLR is prominent in the TIL-low group. Although the majority of ER-positive/HER2-negative breast cancers had low TIL levels

(372 out of 491 cases, 75.8%) (Figure 2), a significant association between NLR levels and OS was obtained in the ER-positive/HER2-negative subtype of the TIL-low group. The association between TIL levels and the prognostic impact

Table III. Univariate and multivariate analyses of overall survival in the ER-positive/HER2-negative with TIL-low group.

	n	Univariate analysis HR (95% CI) ^a	p-Value	Multivariate analysis HR (95% CI) ^a	p-Value
Menopausal status					
Pre-	135	1.00			0.079
Post-	234	3.29 (0.88-21.25)			
Tumour size					
≤2.0 cm	234	1.00			0.056
>2 cm	138	2.92 (0.97-9.68)			
Lymph node metastasis					
Negative	259	1.00			0.067
Positive	111	2.79 (0.93-8.68)			
Tumour grade					
1	277	1.00			0.243
2+3	71	2.13 (0.57-6.76)			
Ki67 expression level ^b					
Low	312	1.00	0.0021	1.00	0.008
High	58	5.90 (1.96-18.73)		4.69 (1.54-14.81)	
Chemotherapy					
No	320	1.00			0.393
Yes	52	0.45 (0.025-2.30)			
NLR level ^c					
Low	264	1.00	0.0067	1.00	0.022
High	108	4.70 (1.53-13.38)		3.78 (1.21-14.17)	

^aHazard ratio (95% confidence interval); ^blow: <25%, high: ≥25%; ^chigh: ≥2.72, low: <2.72.

of NLR still needs to be investigated. The results of this study need to be considered within the context of the study limitations. First, data obtained here were generated by subgroup analyses in a cohort recruited retrospectively at a single institute. Second, we set the cut-off value of NLR at 2.72 obtained by ROC curve (14), and it is unknown whether this is the best optimal cut-off value. Additionally, we compared NLR and TIL levels but not subsets of TILs, including CD8-positive or FOXP3-positive T cells. These factors need to be confirmed in a future study with a larger sample size where the immunohistochemical evaluation of TIL subsets can be investigated.

In conclusion, we have identified that the prognostic significance of NLR is limited to ER-positive/HER2-negative breast cancers with low levels of TIL. Since NLR and TILs seem to have a role in patients' prognosis by mediating different immunological mechanisms, a combination of these factors may be useful not only for selecting patients with poor prognosis in daily clinical practice but also for understanding the mechanisms through which these factors affect patients' prognosis.

Conflicts of Interest

YaM received research funding and honoraria from Chugai, AstraZeneca, Eli Lilly, Pfizer, MSD, Kyowa-Kirin, Taiho, and Esai. The other Authors declare that they have no conflicts of interest.

Authors' Contributions

AB, and YaM designed the study. YF, and TW evaluated expression levels of TIL.

AB, TH, AS, RF, HO, YoM, and MI were involved in data collection. AB, TH, and YaM performed the statistical analyses. AB, and YaM prepared the manuscript. All Authors read and approved the final version of manuscript.

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