Prognostic Significance of Pre-treatment Neutrophil: Lymphocyte Ratio in Japanese Patients with Breast Cancer

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Abstract. *Background: The neutrophil:lymphocyte ratio (NLR) has been reported to reflect systemic inflammation and to have independent prognostic value for patients with various cancers. In this study, we analyzed the association between NLR and clinicopathological factors and verified the significance of NLR as a prognostic factor for Japanese patients with breast cancer. Patients and Methods: A total of 167 Japanese female patients with stage I–III breast cancer were retrospectively recruited into this study. Associations with clinicopathological factors and NLR were assessed, and disease-free survival and breast cancer-specific survival were estimated. Results: In multivariate analysis, lymph node metastases and NLR were significantly associated with disease-free survival and breast cancer-specific survival. NLR was significantly higher in patients with lower body- mass index. Conclusion: Preoperative NLR may be an independent prognostic factor for survival in Japanese patients with breast cancer. Reduction of body mass index has been implicated in NLR elevation, particularly in postmenopausal women.*

In 1863, the German physician Rudolf Carl Virchow described the cellular origins of cancer for the first time and suggested that lymphoreticular infiltrates reflected cancer origins at chronic inflammation sites. Subsequent research supported Virchow's hypothesis, and the ensuing relationships between cancer and inflammation have considerable implications for cancer prevention and treatment. Epidemiological studies estimate that nearly 15% of all cancer cases are associated with microbial infections. In particular, hepatitis B and C viruses and *Helicobacter pylori* are causal agents of hepatocellular carcinomas and gastric cancers, respectively. Similarly, a subset of colon cancer is caused by inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease. In addition, inflammation is necessary to maintain and promote cancer progression by causing tumor tissue remodeling, angiogenesis, metastasis, and by suppressing innate anticancer immune responses (1). The Glasgow Prognostic Score (GPS), which combines C-reactive protein and albumin levels, is a well-known prognostic scoring system for patients with various types of advanced cancer. Proctor *et al*. recently reported that the modified GPS (mGPS) is a more powerful independent prognostic factor in patients with cancer than is the GPS (2). Thus far, approximately 60 reports have demonstrated the prognostic value of GPS/mGPS in more than 30,000 patients with cancer. Consequently it has been established that GPS/mGPS is an independent predictive factor of cancer prognosis (2). Several recent reports used the neutrophil: lymphocyte ratio (NLR) as an alternative to the GPS/mGPS to evaluate systemic inflammation in patients with cancer (3). Associations between NLR and other markers of systemic inflammation in patients with operable cancer are clear, particularly with elevated C-reactive protein and hypoalbuminaemia. However, the significance of each index may vary depending on disease stage and organ. Nonetheless, no clear conclusions of superiority have been drawn. Recently, two groups reported the prognostic value of NLR in patients with breast cancer. Namely, Noh *et al*. concluded that patients with an elevated pre-treatment NLR showed poorer disease-specific survival than those without elevated NLR, particularly patients with the luminal-A subtype breast cancer (4). However, to the best of our knowledge, no previous Japanese reports have demonstrated the prognostic value of NLR in patients with breast cancer. The purpose of this study was to investigate the prognostic value of NLR in Japanese patients with breast cancer and to identify influential clinical factors.

Patients and Methods

Patients. A total of 652 patients with operable breast cancer were treated between 2001 and 2011 at the Hokkaido University Hospital. Patient medical records were reviewed, and medical history, age, height, weight, pathological results, and laboratory data were

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Key Words: Breast cancer, neutrophil:lymphocyte ratio, body-mass index.

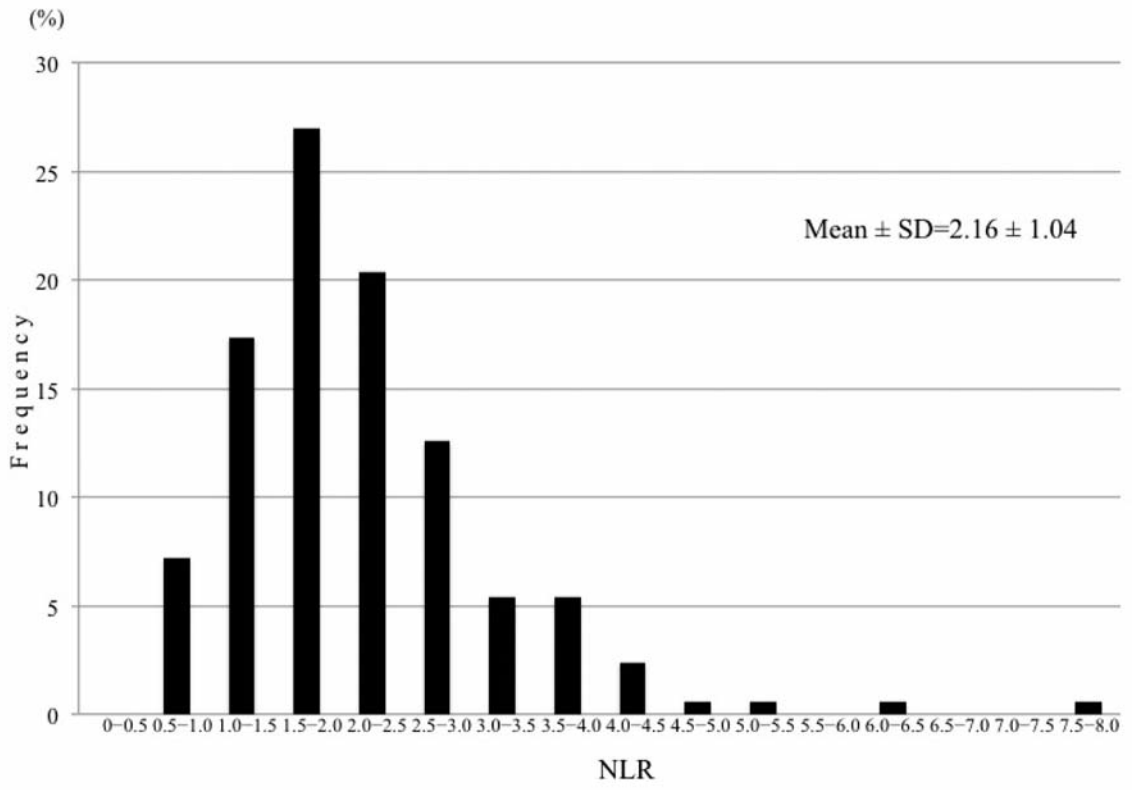

Figure 1. *Histogram of the distribution of neutrophil:lymphocyte ratio (NLR) among 167 patients with breast cancer. The median NLR was 2.16 (range=0.55-7.82).*

retrieved. Patients with ductal carcinoma *in situ* with or without microinvasion and patients lacking pathological or laboratory information were excluded. Patients who had received chemotherapy or immunosuppressive drugs, such as glucocorticoids, ciclosporin, tacrolimus, or interferons, were also excluded. Finally, 167 Japanese female patients with stage I to III breast cancer were recruited. The mean follow-up period was 85.8 months (range=19.8-148.9 months). The present study protocol was approved by the Institutional Review Board (#012-0038) and conforms with the guidelines of the 1996 Declaration of Helsinki.

Pathological characteristics. Pathological data were reviewed to determine tumor size, histological grade, lymph node status (numbers of positive lymph nodes and all lymph nodes, if axillary lymph nodes were dissected), hormone receptor status, human epidermal growth factor receptor 2 (HER2) status, and to retrieve laboratory data. Estrogen receptor (ER) and progesterone receptor (PR) status was evaluated using immunohistochemistry (IHC), and proportions of stained cells and staining intensity were estimated according to Allred's scores. Tumors with total scores of ≥3 were recorded as being positive (5).

Blood parameters. Venous blood samples were taken at the time of diagnosis before neoadjuvant chemotherapy or surgery. NLR was defined as the neutrophil count divided by the lymphocyte count, and NLR cut-off values of ≥2.5 and <2.5 were used according to previous studies (3).

Statistical analysis. Associations between NLR and clinicopathological parameters and clinical outcomes were evaluated in a cross-table using the χ^2 test and an unpaired or Welch's *t*-tests. Correlations were analyzed using Spearman's rank correlation coefficient. Significant differences in disease-free and breast cancer-specific survival were identified using the log-rank test. Differences between patient subgroups were identified using one-way analysis of variance, and the Scheffe test was used for multiple comparisons. Multivariate analysis was performed using the Cox proportional hazards model for each factor. All statistical analyses were performed using StatMate III for Windows v3.18 (ATMS Co., Ltd. Tokyo, Japan) and differences were considered significant when $p<0.05$.

Results

Patients' characteristics. At diagnosis, the distribution of tumor stages I, II, and III were 76 (45.5%), 68 (40.7%), and 23 (13.8%), respectively. The frequency distribution of NLR is shown in Figure 1. The median NLR was 2.16 (range=0.55-7.82).

Blood parameters and clinicopathological factors. Patients with NLR of ≥2.5 were younger and had higher histological grades, higher recurrence rates, and higher mortality than those with NLR of <2.5 (Table I). However, there were no

significant correlations between NLR and clinicopathological factors (data not shown). Therefore, we examined correlations between blood parameters and clinicopathological factors, but observed only weak correlations with body-mass index (BMI, kg/m^2) and total leukocyte counts (Figure 2A). After stratification by menopausal status, these correlations were confirmed only in post-menopausal patients (Figure 2B and C). Patients were subsequently grouped according to BMI of ≥25, 22-25, and <22, and significantly higher NLR values were observed in patients with BMI of <22 (Figure 3A). Subsequent stratification according to menopausal status showed significant associations between BMI and NLR, only in post-menopausal patients (Figure 3B and C).

Prognostic significance of NLR for disease-free and breast cancer-specific survival. The mean NLR of patients with recurrence were significantly higher than that of relapse-free patients (Figure 4). In our study population, patients with NLR of ≥2.5 had a poorer prognosis than those with NLR of <2.5 in univariate analysis for both disease-free and breast cancer-specific survival (Table II). In multivariate analysis, the presence of axillary lymph node metastases were the most accurate predictor of prognosis. A near-significant relationship was observed between NLR and disease-free survival, and NLR was a significant predictor of breast cancer-specific survival (Table II).

Discussion

NLR is a simple marker of sub-clinical inflammation that can be easily assessed using white blood cell counting. Numerous studies have revealed important associations between systemic inflammation and prognosis in patients with various types of cancer and in those with coronary artery disease (3, 6). The prognostic value of the mGPS has been established for several types of cancer. Similarly, NLR has been demonstrated to be an independent prognostic indicator for cancer recurrence and survival (3). Moreover, several studies report factors that are associated with elevated NLR. In particular, clinicopathological factors such as increased tumor size, microvascular and lymphatic invasion, lymph node involvement, and serum concentrations have been associated with elevated NLR (3). Among studies on NLR, 34 report information from patients with operable cancer in five different countries, including Japan (3, 4), and report NLR for cholangiocarcinoma and colorectal, gastric, esophageal, pancreatic, liver, lung, renal, gallbladder, ovarian and breast cancer. However, only recent studies report NLR in patients with operable breast cancer. Noh *et al*. reported poorer breast cancer-specific survival in patients with elevated pre-treatment NLR, particularly in those with luminal A subtypes (4). However, they did not investigate the causes of elevated NLR.

Table I. *Association between neutrophil:lymphocyte ratio (NLR) and clinicopathological factors*.

Characteristic	NLR<2.5 (n=120) No. (%)	NLR≥2.5 (n=47) No. (%)	*p*-Value
Age (years)			
mean±SD	58.9±11.1 (33-84)	55.1±10.1 (33-76)	*0.03*
Menopausal status			
pre-menopause	27 (22.5)	15 (31.9)	
post-menopause	93 (77.5)	32 (68.1)	0.21
Body-mass index			
mean±SD	23.8±3.6	24.0±6.7	0.86
Histology			
IDC	98 (81.7)	43 (91.4)	
ILC	8 (6.7)	2 (4.3)	
Other	14 (11.6)	2 (4.3)	0.27
Stage			
I	56 (46.7)	20 (42.6)	
II	52 (43.3)	16 (34.0)	
III	12 (10)	11 (23.4)	0.07
T-Stage			
T1	62 (51.7)	21 (44.7)	
T2	42 (35)	19 (40.4)	
T3	9 (7.5)	2 (4.3)	
T4	7 (5.8)	5 (10.6)	0.53
N-Stage			
N0	100 (83.3)	33 (70.3)	
N1	16 (13.3)	9 (19.1)	
N2	2 (1.7)	4 (8.5)	
N3	2 (1.7)	1 (2.1)	0.11
Lymph node metastasis			
0	87 (72.5)	28 (59.6)	
1-3	20 (16.6)	9 (19.1)	
≤4	11 (9.2)	7 (14.9)	0.38
Unknown	2 (1.7)	3 (6.4)	
Estrogen receptor			
Negative	24 (20)	13 (27.7)	
Positive	96 (80)	34 (72.3)	0.28
Progesterone receptor			
Negative	52 (43.3)	22 (46.8)	
Positive	68 (56.7)	25 (53.2)	0.68
HER2			
Negative	81 (67.5)	32 (68.1)	
Positive	17 (14.2)	7 (14.9)	0.93
Unknown	22 (18.3)	8 (17.0)	
Histological grade			
1	34 (28.3)	12 (25.5)	
2	69 (57.5)	18 (38.3)	
3	17 (14.2)	17 (36.2)	*0.005*
Recurrence			
Yes	19 (15.8)	16 (34.0)	
Locoregional	4 (3.3)	2 (4.3)	
Distant	15 (12.5)	14 (29.7)	
No	101 (84.2)	31 (66.0)	*0.009*
Death			
Yes	9 (7.5)	10 (21.3)	
No	111 (92.5)	37 (78.7)	*0.01*

IDC: Invasive ductal carcinoma; ILC: invasive lobular carcinoma; HER2: human epidermal growth factor. Values of $p<0.05$ were considered significant. Statistically significant differences are indicated in italics.

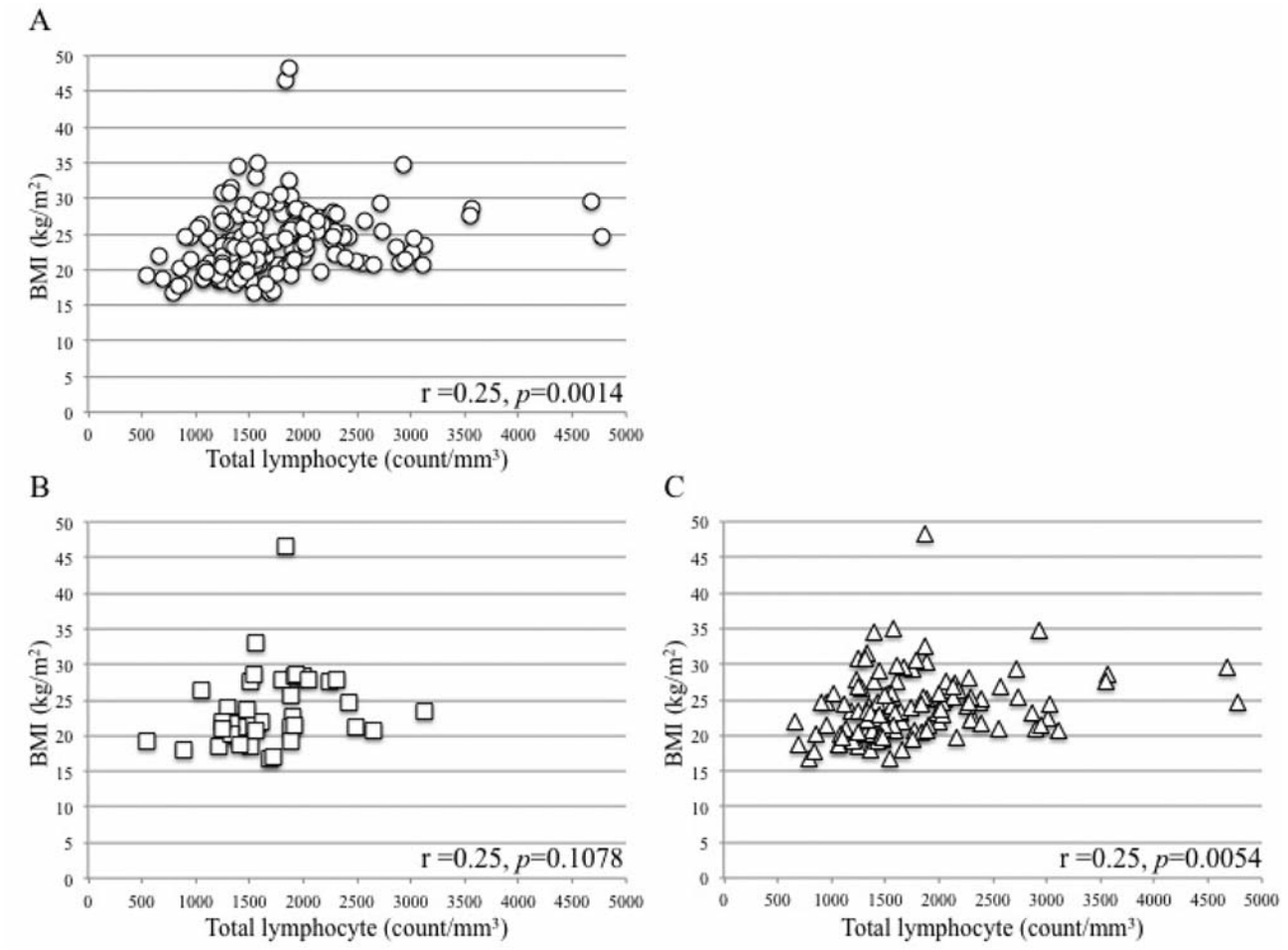

Figure 2. *Correlation between body mass index (BMI) and total lymphocyte counts in all 167 patients (A), 42 premenopausal patients (B), 125 postmenopausal patients (C); differences were considered significant when p<0.05; r, coefficient of correlation.*

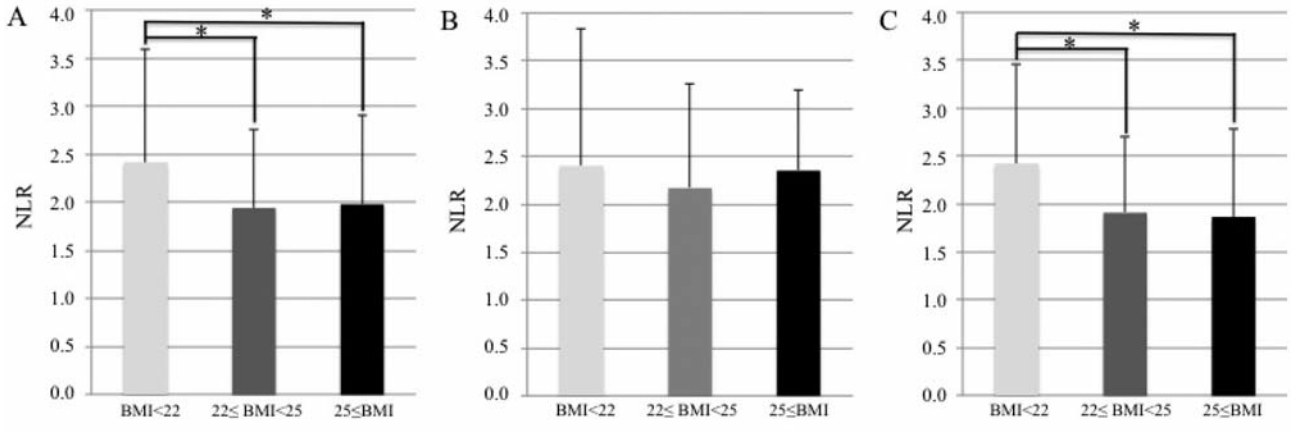

Figure 3. *Neutrophil:lymphocyte ratio (NLR) across body mass index (BMI) groups for all 167 patients (A), 42 premenopausal patients (B), 125 postmenopausal patients (C). A significant difference was observed between patients with BMI of <22 and those with BMI of ≥25, or 22-25 among all 167 patients and 125 postmenopausal patients. Data are presented as the mean±SD. Differences between BMI groups were identified using post hoc tests. *p<0.05.*

Table II. *Univariate and multivariate analyses of clinicopathological factors and neutrophil:lymphocyte ratio (NLR) for disease-free survival and breast cancer-specific survival.*

Clinicopathological parameter		Disease-free survival				Breast cancer-specific survival			
		Univariate		Multivariate		Univariate		Multivariate	
Characteristic	Status	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Menopausal status	Premenopausal *vs*. postmenopausal	1.2 (0.5-2.5)	0.69			0.8 (0.3-2.2)	0.621		
ER	Negative *vs*. positive	1.1 (0.5-2.5)	0.818			2.4 (0.9-9.0)	0.066		
T-Stage	T2-4 *vs*. T1	2.7 (1.3-5.1)	0.005	1.2 (0.8-3.8)	0.62	3.0 (1.1-6.8)	0.027		
Lymph node metastasis	Positive *vs*. negative	4.7 (3.2-14.3)	<0.001	4.5 (1.9-10.2)	*<0.001*	7.7 (4.0-31.0)	<0.001	9.4 (2.8-31.6)	*<0.001*
HG	3 *vs*. 1,2	2.8 (1.6-9.3)	0.002	1.8 (0.8-3.8)	0.14	7.6 (6.2-63.0)	<0.001		
NLR	NLR ≥2.5 *vs*. NLR <2.5	2.5 (1.4-6.4)	0.004	2.0 (0.9-4.1)	0.07	3.2 (1.5-11.5)	0.007	2.7 (1.1-7.3)	*0.045*

ER: Estrogen receptor; HG: histological stage; NLR: HR: hazard ratio; CI, confidence interval. Values of $p<0.05$ were considered significant. Statistically significant differences are indicated in italics.

In cross-sectional studies, higher BMI is consistently linked with elevated endogenous estrogen levels (7-11). Moreover, increasing BMI is reportedly associated with elevated total estradiol in postmenopausal women, but is associated with decreased estradiol in pre-menopausal women, particularly during the follicular phase (12-14). Jones *et al*. reported that gradual changes in BMI and plasma leptin levels in post-menopausal women are also associated with changes in estradiol and testosterone levels (15). Estrogens inhibit bone resorption and inflammation in patients with chronic inflammatory disease, they support immunity in trauma/sepsis and exhibit pro-inflammatory effects in some chronic autoimmune diseases (16).

In the present study, a weakly-positive correlation was observed between BMI and total leukocyte counts. In addition, patients with breast cancer with lower BMI had significantly higher NLR. Being severely underweight is a risk factor for low bone mineral density, anemia, and amenorrhea, and the associated malnutrition tends to reduce protein nutrition, serum albumin, and lymphocyte counts. Earlier studies reported a decreased lymphocyte count in patients with anorexia nervosa who were severely underweight (17, 18).

In conclusion, a high preoperative NLR was associated with poor prognosis in patients with breast cancer, and postmenopausal women with a low BMI may tend to have higher NLR. However, further studies are required to better understand the role of pretreatment NLR value.

Conflicts of Interest

The Authors declare that they have no competing interests.

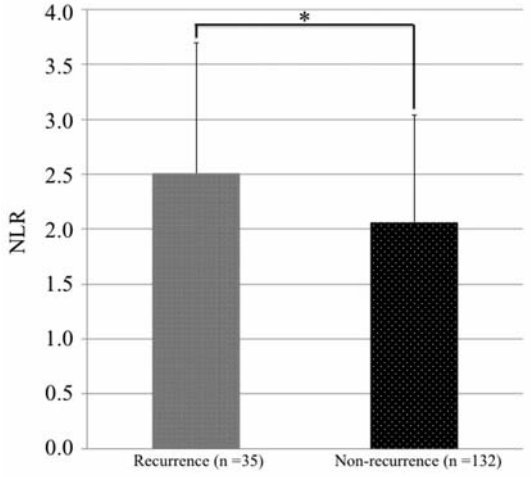

Figure 4. *Comparison of neutrophil:lymphocyte ratio (NLR) between patients with non-recurrent and recurrent breast cancer. Data are presented as the mean±SD. *p<0.05.*

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