Neutrophil-to-lymphocyte ratio before each chemotherapy line predicts clinical outcomes in patients with unresectable gastric cancer

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Abstract. The neutrophil-to-lymphocyte ratio (NLR) is a well-known prognostic biomarker for patients with gastric cancer (GC). However, for patients with GC treated with palliative chemotherapy, the predictive values of NLR remain obscure. Therefore, the present study evaluated the clinical impact of NLR in patients with GC treated with a series of chemotherapies. The present study retrospectively evaluated 83 patients with unresectable GC who received a series of chemotherapies. NLR in the blood was calculated before each chemotherapy initiation (before 1st-, 2nd- and 3rd-line treatment). Of the 83 patients enrolled, 56 patients (67%) received 2nd-line chemotherapy and 34 patients (41%) received 3rd-line chemotherapy. NLR at 1st-line ranged from 0.72 to 48.9 (median NLR, 3.00). Therefore, the median NLR of 3.00 was used as a definite cut-off value throughout the present study. All patients were dichotomized into NLR-high (>3.00) and NLR-low group (<3.00) by NLR evaluated before each line of chemotherapy. The median overall survival (OS) time of the low-NLR group was better than that of the high-NLR group from 1st-line to 3rd-line treatment (1st-line: 18.1 vs. 8.0 months, P=0.06; 2nd-line: 10.7 vs. 4.5 months, P=0.0001; 3rd-line: 8.7 vs. 4.7 months, P=0.003). Of the 24 patients treated with 3rd-line nivolumab, patients with low NLR exhibited better OS than those with high NLR (8.3 months in low-NLR and 6.6 months in high-NLR, P=0.06). In conclusion, NLR should be performed before each chemotherapy line in the clinical setting and may predict outcomes in patients with unresectable GC, including those treated with nivolumab.

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Introduction

Gastric cancer (GC) is the fifth most common cancer and the third most common cause of cancer-related mortality worldwide (1). Systemic chemotherapy, including cytotoxic agents, molecular-targeted drugs, or immune checkpoint inhibitors, has demonstrated a statistically significant prolongation of survival compared with best supportive care alone. Therefore, it is recommended as a first-line or later conventional treatment for advanced GC (2-4). In addition to other cancers, blockade of program death-1 (PD-1) or program death ligand-1 (PD-L1) restores T-cell activity and has emerged as a breakthrough therapy for GC.

Among the specific drugs, nivolumab, a PD-1 inhibitor, was evaluated for its efficacy against GC in a randomized phase III study, ATTRACTION-2 (5). The ATTRACTION-2 results indicated that patients treated with nivolumab exhibited significantly prolonged OS compared with those treated with placebo. Although this represents an advancement in GC treatment, the outcome of patients with unresectable GC remains poor. Indeed, the median OS is approximately one year after initiation of first-line chemotherapy (6). In the ATTRACTION-2 study, the patients receiving nivolumab showed a significant increase in median OS; however, approximately half experienced exhibited progressive disease at their first radiographical examination. Thus, biomarkers that can predict the outcome or efficacy of these agents are needed.

Systemic inflammation is vital in tumor promotion and progression (7). Several markers of systemic inflammation, including C reactive protein, platelet-to-lymphocyte ratio, and neutrophil-to-lymphocyte ratio (NLR), are associated with clinical outcomes in various cancers. The peripheral blood NLR, an indicator of systemic inflammation, is a conventional biomarker in the clinical setting and has been reported as a prognostic biomarker in solid neoplasms, such as breast, lung, pancreas, colon, rectum, and stomach (8-12). In addition to its prognostic role, NLR has been recognized as a predictive marker for several unresectable solid neoplasm treatments with cytotoxic chemotherapy and immunotherapy (13,14). Recently, Valero *et al* demonstrated that increased NLR is significantly associated with poorer OS and progression-free survival and lower response rates and clinical benefit after immune

checkpoint inhibitor (ICI) therapy for multiple cancer types in a retrospective cohort of 1,714 patients representing 16 different cancer types, including GC patients treated with ICI (15).

Although the NLR values fluctuate over time, most studies have used NLR values obtained at a one-time point before the induction of targeted agent therapy to evaluate clinical outcomes. In this study, we evaluated NLR values in blood at the moment before the initiation of each chemotherapy line (e.g., before the 1st-, 2nd-, and 3rd-line, respectively) to determine whether NLR could predict clinical outcome.

Materials and methods

Patients. In this retrospective study, we analyzed 83 patients with unresectable GC who were histologically diagnosed with adenocarcinoma and received palliative chemotherapy in the Department of Clinical Oncology, Kawasaki Medical School Hospital, between March 2018 and December 2020. All patients-received one or more lines of chemotherapy and at least one evaluation of anti-tumor efficacy by computed tomography after the start of chemotherapy. The duration of follow-up ranged from 1.0 to 58.4 months (median: 10.1 months). OS was calculated from the initiation of each line of chemotherapy to death. Clinicopathological factors, e.g., age at the start of chemotherapy, gender, an Eastern Cooperative Oncology Group (ECOG) performance status, primary tumor location, human epidermal growth factor receptor type 2 (HER2) status, histology, metastatic status, and chemotherapy regimens, were collected from clinical and pathological records. The HER2 status was evaluated using immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). Tumors with IHC 3+, IHC 2+, or FISH positive were HER2 positive (16). According to the Japanese classification of gastric carcinoma (17), the primary tumor location was classified into three categories (upper, middle, and lower). GCs were classified according to Lauren's classification (18). Metastatic sites were evaluated by computed tomography, positron emission tomography, or magnetic resonance imaging, which were examined before induction of each chemotherapy.

Chemotherapy. All patients received 1st-line chemotherapy (e.g., SOX, XELOX, FOLFOX SP, XP, weekly solvent-based paclitaxel (sb-paclitaxel) plus ramucirumab, weekly nab-paclitaxel plus ramucirumab, docetaxel monotherapy, or S-1 monotherapy) according to the Japanese GC treatment guidelines. Details of the number of patients using each regimen are presented in Table I. These regimens were administered as follows: i) SOX: twice daily for the first 2 weeks of a 3-week cycle of S-1 (80-120 mg/day) with 100 mg/m^2 of oxaliplatin on day one; ii) XELOX: twice daily for the first 2 weeks of a 3-week cycle of capecitabine (2,400 to 4,200 mg/day) with 130 mg/m^2 of oxaliplatin on day one; iii) FOLFOX: oxaliplatin 85 mg/m^2 and LV 200 mg/m^2 followed by bolus 5-FU 400 mg/m^2 and continuous 5-FU 2,400 mg/m^2 were intravenously infused every 2 weeks; iv) SP regimen: twice daily for the first 3 weeks of a 5-week cycle of S-1 (80-120 mg/day) with 60 mg/m^2 of cisplatin on day 8 of each cycle; v) XP regimen: twice daily for the first 2 weeks of a 3-week cycle of capecitabine (2,400-4,200 mg/day) with 100 mg/m^2 of cisplatin on day one; vi) Sb-paclitaxel plus ramucirumab regimen: ramucirumab (8 mg/kg intravenously on days 1 and 15) with sb-paclitaxel (80 mg/m^2 intravenously on days 1, 8, and 15) every 4 weeks. vii) Nab-paclitaxel plus ramucirumab regimen: Ramucirumab (8 mg/kg intravenously on days 1 and 15) with nab-paclitaxel (100 mg/m^2 intravenously on days 1, 8, and 15) every 4 weeks; and viii) Docetaxel regimen: Docetaxel (60-70 mg/m^2) was administered intravenously on day 1 every 3 weeks. ix) S-1 monotherapy: Twice daily for the first 2 weeks of a 3-week cycle of S-1 (80-120 mg/day). Dose reduction and/or cycle delays were permitted according to the decision of each physician. Fifteen patients that were HER2-positive received trastuzumab in combination with the SOX or XP regimen.

This study summarized i) SOX, ii) XELOX, iii) FOLFOX, iv) SP, and v) XP regimens as Platinum-based chemotherapy regimens. The vi) weekly sb-paclitaxel plus ramucirumab, vii) weekly nab-paclitaxel plus ramucirumab, and viii) docetaxel monotherapy were Taxane-based chemotherapy regimens. The ix) S1 monotherapy was categorized as others. S1 monotherapy is sometimes used in the front-line chemotherapy in Japan. Although S1 monotherapy is restricted to use to the patients with PS 3, we introduced intensive chemotherapy to the patients with PS 3 when we judged that PS was getting worse due to stomach cancer burden.

Evaluation of NLR in blood. NLR was calculated by dividing the absolute neutrophil and lymphocyte counts measured in peripheral blood before each line of chemotherapy. Previous studies varied the cut-off value of NLR from 2.0 to 5.0. A meta-analysis summarized the median NLR for OS, cancer-specific OS, and progression-free survival as 4.0, 3.85, and 3.0, respectively (19). Moreover, the meta-analysis revealed a consistent effect of an elevated NLR on survival. As the median NLR at 1st-line initiation in this study was 3.0 (range: 0.7 to 48.9), we used the lowest NLR cut-off value of 3.0 among OS, cancer-specific OS, and progression-free survival in the meta-analysis (19) as the cut-off value throughout the study. Patients were divided into an NLR-high group (>3.0) and an NLR-low group (<3.0) based on the NLR value determined before the initiation of each line of chemotherapy.

Statistical analyses. The primary aim was to evaluate the association between peripheral blood NLR and clinical outcomes in GC patients. OS was calculated using the Kaplan-Meier method, and the log-rank test was used to compare survival between groups. Fisher's exact test was performed to compare clinical characteristics between two groups. Logistic regression analysis was used to obtain the risk ratio of the NLR-high group to the NLR-low group. Cox regression analysis was used to estimate the hazard ratio (HR) for OS. All statistical tests were two-sided, and $P<0.05$ was considered statistically significant. All statistical analyses were performed with EZR software (Saitama Medical Centre, Jichi Medical University, Saitama, Japan, version 1.40), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, version 3.5.2) (20).

Results

Patient characteristics. A total of 83 patients were enrolled. Patient characteristics are shown in Table I. The median age

Table I. Clinicopathological characteristics of patients with unresectable GC patients.

Characteristic	Line of chemotherapy		
	1st-line (n=83)	2nd-line (n=56)	3rd-line (n=34)
Sex, n (%)			
Male	52 (63)	34 (61)	20 (59)
Female	31 (37)	22 (39)	14 (41)
Median age, years (range)	72 (44-86)	72 (53-79)	70 (53-77)
Age, n (%)			
<70 years	36 (43)	28 (50)	16 (47)
≥70 years	47 (57)	28 (50)	18 (53)
Performance status, n (%)			
0	25 (30)	15 (27)	7 (21)
1	30 (36)	22 (40)	16 (47)
2	22 (27)	16 (28)	10 (29)
3	6 (7)	3 (5)	1 (3)
Primary tumor location, n (%)			
Upper	29 (35)	17 (30)	12 (35)
Middle	34 (41)	20 (36)	13 (38)
Lower	20 (24)	19 (34)	9 (27)
HER2 status, n (%)			
Positive	15 (18)	11 (20)	7 (21)
Negative	68 (72)	45 (80)	27 (79)
Lauren classification, n (%)			
Intestinal type	45 (54)	30 (54)	16 (47)
Diffuse type	38 (46)	26 (46)	18 (53)
Number of metastatic organs, n (%)			
≤1	51 (61)	27 (48)	17 (50)
≥2	32 (39)	29 (52)	17 (50)
Liver metastasis, n (%)			
Yes	26 (31)	21 (38)	10 (29)
No	57 (69)	35 (62)	24 (71)
Peritoneal dissemination, n (%)			
Yes	35 (42)	23 (41)	14 (41)
No	48 (58)	33 (59)	20 (59)
Ascites, n (%)			
Yes	30 (36)	13 (23)	14 (41)
No	53 (64)	43 (77)	20 (59)
Chemotherapy regimen, n (%)			
Platinum-based			
No antibody agent	61 (73)	4 (7)	0 (0)
Trastuzumab	13 (16)	0 (0)	0 (0)
Taxane-based	5 (6)	48 (85)	3 (9)
ICI	0 (0)	2 (4)	24 (70)
Others	4 (5)	2 (4)	7 (21)

Data are presented as median (range) or n (%). ICI, immune checkpoint inhibitor.

was 72 (range: 44-86), and 63% (n=52) of the patients were male. Fifty-five patients (66%) had good performance status (ECOG performance status: 0-1). Among six patients with PS 3, four patients received S-1 monotherapy. Thirty-two patients (39%) had two or more metastatic organs, 26 (31%) had liver metastases, and 35 (42%) confirmed peritoneal dissemination. Based on the cut-off value, at the induction of the 1st-line chemotherapy, 42 patients were categorized

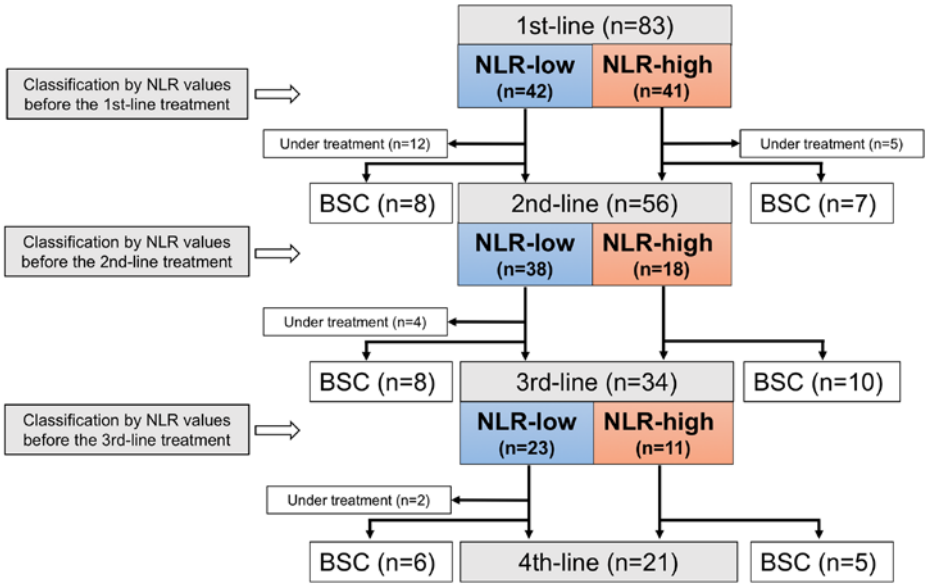


Figure 1. The participant flow diagram of the GC patient cohort. Treatment progress according to classification by pretreatment NLR for each line. GC, gastric cancer; NLR, neutrophil-to-lymphocyte ratio; BSC, best supportive care.

into the NLR-low group (NLR <3.0) and 41 patients into the NLR-high group (NLR ≥3.0; Fig. 1).

At the analysis, 12 patients were still undergoing the 1st-line treatment, 15 were transferred to best supportive care (BSC) after the 1st-line treatment, and 56 (79%) received the 2nd-line treatment. Of the 56 patients, 38 and 18 were divided into the NLR-low and NLR-high groups by blood NLRs before the 2nd-line treatment.

Among the 2nd-line treatment patients, four were still undergoing the 2nd-line treatment at the analysis, and 34 (65%) had received the 3rd-line treatment. Of the 34 patients, 23 and 11 were divided into the NLR-low and NLR-high groups by blood NLRs before the 3rd-line treatment. Finally, of the 34 patients treated with the 3rd-line chemotherapy, two were still under the 3rd-line treatment at the analysis, 21 (66%) received the 4th-line chemotherapy, and the remaining 11 patients were transferred to BSC.

Clinical outcomes in relation to NLR values before each chemotherapy line. Table II presents the clinicopathological characteristics of GC patients at each chemotherapy line concerning the NLR-high and NLR-low groups. Regarding the patient characteristics, the NLR-high group had significantly more patients with a poor ECOG performance status throughout chemotherapy.

Of all 83 patients, the median OS was 13.2 months (95% CI: 9.1-17.9). The median OS from the start of the 1st-line chemotherapy was shorter in the NLR-high group than in the NLR-low group [OS: 8.0 months (95% CI: 5.7-13.2) vs. 18.1 months (11.9-20.9), P=0.06; Fig. 2A]. Of 56 patients who received 2nd-line chemotherapy, the median OS from the start of the 2nd-line chemotherapy was significantly shorter in the NLR-high group than in the NLR-low group [OS: 4.5 months (95% CI: 3.4-6.0) vs. 10.7 months (95% CI: 7.4-13.7), P<0.05; Fig. 2B]. Of the 34 patients who received 3rd-line chemotherapy, the median OS from the start of the 3rd-line chemotherapy was significantly shorter in the NLR-high group than in the NLR-low group [OS: 4.7 months (95% CI: 0.8-7.3) vs. 8.7 months (95% CI: 5.6-14.0), P<0.05; Fig. 2C].

Next, we focused on the 24 patients who received nivolumab monotherapy at the 3rd-line treatment. According to the NLR value before nivolumab therapy, 14 patients (55%) were stratified into the NLR-low group and ten patients into the NLR-high group. Patient characteristics are shown in Table SI. The median OS was shorter in the NLR-high group than that in the NLR-low group [median OS: 6.6 months (95% CI: 0.8-7.3 months) vs. 8.3 months (2.6-10.3 months), P=0.06; Fig. 2D].

Finally, we examined which factor affected NLR values in each chemotherapy line by logistic regression analysis (Table III). By this multivariate analysis, poor ECOG performance status was significantly associated with the NLR-high group throughout the 1st- to 3rd-line. In contrast, regarding prognosis, Cox regression analysis revealed that the NLR high group was significantly associated with poor prognosis only in the 3rd-line OS (risk ratio=4.33, P=0.03; Table IV).

Changes in NLR value throughout chemotherapeutic drug treatment in unresectable GC patients. We examined fluctuations of NLR values in each case throughout chemotherapy (Fig. S1). Among 35 patients in the NLR-low group at 1st-line who were eligible for 2nd-line treatment, 23 (66%) retained their NLR within 3.0, and only 4 cases (13%) showed an increase in NLR by 3.0 or more. In contrast, of 36 patients in the NLR-high group at 1st-line who were eligible for 2nd-line treatment, 15 patients (42%) recovered their NLR within 3.0, but 14 (39%) retained the NLR as 3.0 or more at the initiation of the 2nd-line therapy (P=0.026; Fig. 3).

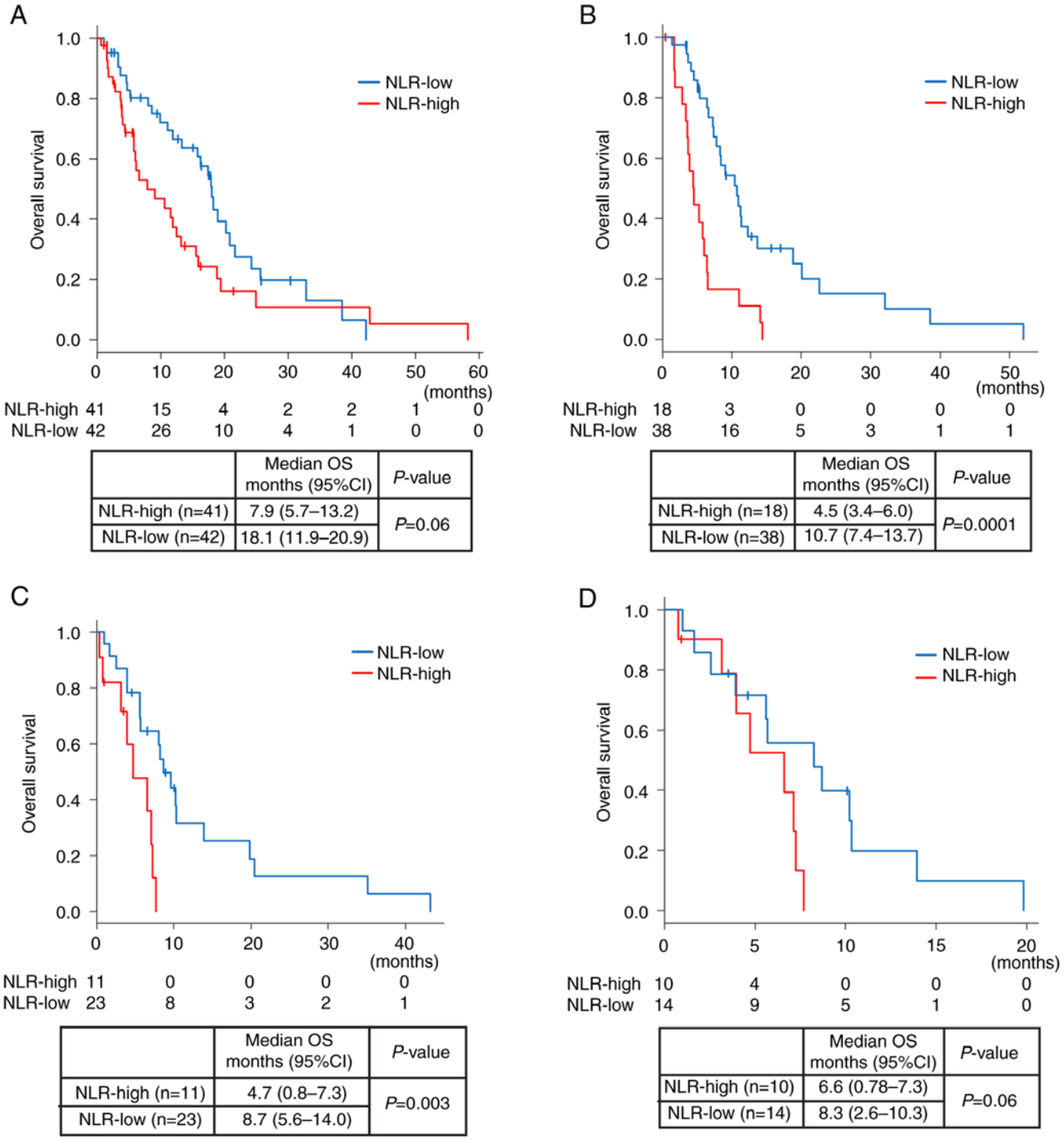
Discussion

This study examined whether the NLR value in blood obtained before initiation of the 1st-, 2nd-, and 3rd-line chemotherapy could represent a prognostic biomarker for unresectable GC.

Table II. The association between clinicopathological characteristics of unresectable GC patients and NLR variations obtained before the 1st-, 2nd-, and 3rd-line chemotherapy.

	NLR classification before each initiation for chemotherapy, n (%)								
	1st-line			2nd-line			3rd-line		
Characteristics	NLR Low (N=42)	High (N=41)	P-value	NLR Low (N=38)	High (N=18)	P-value	NLR Low (N=23)	High (N=11)	P-value
Sex			0.82			0.77			0.46
Male	27 (64)	25 (61)		24 (63)	10 (56)		15 (65)	5 (46)	
Female	15 (36)	16 (39)		14 (37)	8 (44)		8 (35)	6 (54)	
Age			0.38			0.39			0.27
<70 years	16 (38)	20 (49)		21 (55)	7 (39)		9 (39)	7 (64)	
≥70 years	26 (62)	21 (51)		17 (45)	11 (61)		14 (61)	4 (36)	
ECOG PS			0.03			<0.001			0.02
0	15 (36)	10 (24)		14 (37)	1 (6)		6 (26)	1 (9)	
1	19 (45)	11 (27)		18 (47)	4 (22)		13 (57)	3 (27)	
2	7 (17)	15 (37)		5 (13)	11 (61)		3 (13)	7 (64)	
3	1 (2)	5 (12)		1 (3)	2 (11)		1 (4)	0 (0)	
Primary tumor location			0.41			0.16			0.08
Upper	14 (33)	15 (37)		10 (26)	7 (39)		5 (22)	7 (64)	
Middle	20 (48)	14 (34)		12 (32)	8 (44)		10 (44)	3 (27)	
Low	8 (19)	12 (29)		16 (42)	3 (17)		8 (34)	1 (9)	
HER2 status			0.78			0.73			0.38
Positive	7 (17)	8 (20)		7 (18)	4 (22)		6 (26)	1 (9)	
Negative	35 (83)	33 (80)		31(82)	14 (78)		17 (74)	10 (91)	
Lauren classification			1.00			0.40			0.72
Intestinal type	23 (55)	22 (54)		22 (58)	8 (44)		10 (44)	6 (54)	
Diffuse type	19 (45)	19 (46)		16 (42)	10 (56)		13 (56)	5 (46)	
Number of metastatic organs			0.01			0.16			1.00
≤1	32 (76)	19 (46)		21 (55)	6 (33)		11 (48)	6 (55)	
≥2	10 (24)	22 (54)		17 (45)	12 (67)		12 (52)	5 (45)	
Liver metastasis			0.35			0.56			1.00
Yes	11 (26)	15 (37)		13 (34)	8 (44)		6 (30)	4 (36)	
No	31 (74)	26 (63)		25 (66)	10 (56)		14 (70)	7 (64)	
Peritoneal dissemination			0.27			0.04			0.69
Yes	15 (36)	20 (49)		12 (32)	11 (61)		6 (26)	4 (36)	
No	27 (64)	21 (51)		26 (68)	7 (39)		17 (74)	7 (64)	
Ascites			0.02			0.09			0.08
Yes	10 (24)	20 (49)		6 (16)	7 (39)		12 (52)	2 (18)	
No	32 (76)	21 (51)		32 (84)	11 (61)		11 (48)	9 (82)	

Several predictive and prognostic factors were recently evaluated in various cancer types to accurately define patient groups that may benefit from anticancer therapy and predict their survival. As systemic inflammation plays an essential role in tumor promotion and progression (7), systemic inflammatory markers, including C-reactive protein, Platelet-to-Lymphocyte ratio, and NLR, have attracted attention as putative prognostic markers in various cancer types (10-12). Peripheral blood NLR, an indicator of systemic inflammation, is a simple and conventional biomarker that has been considered a prognostic factor in multiple solid neoplasms, including GC (9,21). According to a recent report by Zhou *et al*, NLR was a significant independent prognostic factor for progression-free survival (PFS) and OS, and elevated NLR was associated with poor PFS and OS in unresectable GC patients treated with first-line chemotherapy (22). However, in this study, a multivariate analysis revealed that only 3rd-line pretreatment NLR was associated with the clinical outcomes. Additionally, as shown in Fig 2, the Kaplan-Mayer's curves estimated from the start of the 2nd-line and the 3rd-line demonstrated that the survival curves



	Median OS months (95%CI)	*P*-value
NLR-high (n=41)	7.9 (5.7–13.2)	*P*=0.06
NLR-low (n=42)	18.1 (11.9–20.9)	

	Median OS months (95%CI)	*P*-value
NLR-high (n=18)	4.5 (3.4–6.0)	*P*=0.0001
NLR-low (n=38)	10.7 (7.4–13.7)	

	Median OS months (95%CI)	*P*-value
NLR-high (n=11)	4.7 (0.8–7.3)	*P*=0.003
NLR-low (n=23)	8.7 (5.6–14.0)	

	Median OS months (95%CI)	*P*-value
NLR-high (n=10)	6.6 (0.78–7.3)	*P*=0.06
NLR-low (n=14)	8.3 (2.6–10.3)	

Figure 2. Kaplan-Meier curves of OS according to NLR. Kaplan-Meier analysis for (A) 1st-line OS according to 1st-line pretreatment NLR, (B) for 2nd-line OS according to 2nd-line pretreatment NLR, and (C) for 3rd-line OS according to 3rd-line pretreatment NLR. (D) Kaplan-Meier curves for OS of patients who received nivolumab monotherapy for 3rd-line chemotherapy according to NLR. OS, overall survival; NLR, neutrophil-to-lymphocyte ratio.

by the NLR-low group were significantly better than those by the NLR-high group. This finding may be due to the NLR-high group at late lines possessed more patients with poor PS than the NLR-low groups.

A series of studies measured NLR before initiating the 1st-line chemotherapy (22,23), but NLR is not a fixed value and fluctuates with the patient's condition. Wang *et al* reported that the OS of unresectable GC patients whose NLR levels increased after the 1st-line treatment was nine months, whereas that of patients with decreased NLR was 20 months (24). Changes in NLR following chemotherapy were reported to predict prognosis in many types of carcinomas (24-26). Our results also demonstrate that the mean NLR value significantly decreased in patients who were transferred to the 2nd-line treatment, whereas it increased in patients who could not be transferred to the 2nd-line treatment. Together with another result in this study that, throughout the 1st- to 3rd- line treatment, patients with each NLR-high (>3.0) value were associated with poor EGOG performance status, NLR might be an objective surrogate marker for EGOG performance status.

Table III. Logistic regression analysis estimates the risk ratio for NLR-high among explanatory clinical variables.

	1st-line pretreatment NLR-high		2nd-line pretreatment NLR-high		3rd-line pretreatment NLR-high	
Characteristic	Risk ratio (95%CI)	P-value	Risk ratio (95%CI)	P-value	Risk ratio (95%CI)	P-value
Sex (Male/Female)	0.84 (0.29-2.38)	0.74	1.34 (0.26-6.85)	0.72	0.06 (0.01-1.36)	0.08
Age (≥70/<70)	0.46 (0.16-1.32)	0.15	1.36 (0.22-8.33)	0.74	0.30 (0.02-4.41)	0.38
Performance status (≥2/≤1)	5.17 (1.59-16.8)	0.01[a]	19.2 (2.76-133.6)	<0.01[a]	70.7 (1.98-2526.4)	0.02[a]
HER2 status (Positive/Negative)	1.40 (0.33-5.87)	0.64	1.06 (0.08-13.5)	0.96	0.007 (2.27e-5-2.59)	0.10
Lauren classification (Diffuse/Intestinal)	0.92 (0.31-2.68)	0.87	7.11 (0.91-55.6)	0.06	0.09 (0.004-1.87)	0.12
Number of metastatic organs (≥2/≤1)	3.64 (1.14-11.6)	0.03[a]	1.57 (0.24-10.2)	0.64	0.45 (0.03-7.49)	0.58
Liver metastasis (Yes/No)	1.68 (0.48-5.92)	0.42	3.77 (0.41-34.9)	0.24	1.82 (0.12-26.5)	0.66
Peritoneal dissemination (Yes/No)	1.92 (0.45-8.20)	0.38	5.43 (0.47-62.5)	0.17	1.29 (0.04-39.2)	0.88
Ascites (Yes/No)	0.67 (0.14-3.06)	0.60	1.04 (0.09-11.6)	0.97	0.02 (0.00-1.62)	0.08

[a]P<0.05.

Table IV. Cox regression analyses estimate the Hazard ratio for OS among explanatory clinical variables.

	1st-line OS		2nd-line OS		3rd-line OS	
Characteristic	HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
Sex (Male/Female)	1.44 (0.79-2.61)	0.23	1.52 (0.74-3.09)	0.26	3.05 (0.80-11.7)	0.10
Age (≥70/<70)	1.31 (0.71-2.42)	0.38	0.57 (0.25-1.31)	0.19	0.93 (0.36-2.42)	0.89
Performance status (≥2/≤1)	2.91 (1.52-5.29)	<0.01[a]	6.30 (2.34-16.9)	<0.001[a]	4.57 (0.90-23.1)	0.06
HER2 status (Positive/Negative)	1.14 (0.54-2.42)	0.72	0.69 (0.27-1.80)	0.46	1.18 (0.25-5.50)	0.84
Lauren classification (Diffuse/Intestinal)	1.02 (0.54-1.93)	0.94	0.85 (0.41-1.73)	0.67	6.72 (1.59-28.4)	<0.01[a]
Number of metastatic organs (≥2/≤1)	1.15 (0.56-2.38)	0.70	1.39 (0.62-3.14)	0.42	2.79 (1.00-7.74)	0.04[a]
Liver metastasis (Yes/No)	0.65 (0.29-1.41)	0.27	0.76 (0.32-1.72)	0.50	1.83 (0.57-5.87)	0.31
Peritoneal dissemination (Yes/No)	0.60 (0.27-1.31)	0.20	0.79 (0.31-2.02)	0.62	0.35 (0.08-1.47)	0.15
Ascites (Yes/No)	2.87 (1.21-6.80)	0.02[a]	0.72 (0.22-2.36)	0.59	3.73 (0.86-16.2)	0.08
1st-line pretreatment NLR (High/Low)	1.65 (0.85-3.20)	0.14				
2nd-line pretreatment NLR (High/Low)			2.35 (0.99-5.61)	0.05		
3rd-line pretreatment NLR (High/Low)					5.28 (1.43-19.6)	0.01[a]

HR, hazard ratio. [a]P<0.05.

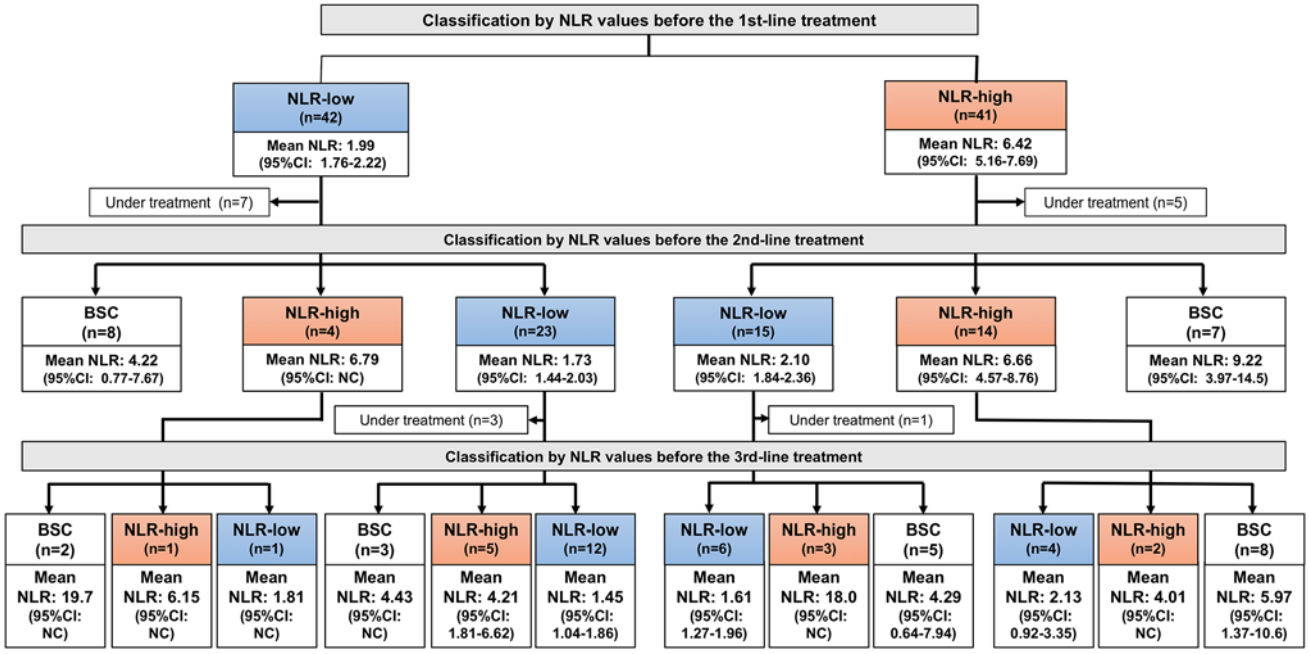


Figure 3. Precise treatment progress according to classification by pretreatment NLR for each line. Mean NLR value and 95%CI are listed under the column. NLR, neutrophil-to-lymphocyte ratio; CI, confidence interval; NC, not calculated.

High NLR was associated with poor prognosis at the 3rd-line by multivariate analysis in this study. Thus, we focused on 3rd-line nivolumab monotherapy patients, consisting of 70% of patients who received 3rd-line chemotherapy. Nivolumab, a PD-1 inhibitor, was evaluated in a randomized phase III study in GC patients with >2 prior chemotherapy regimens and significantly prolonged OS compared with the placebo group (5). However, approximately half of the patients treated with nivolumab did not receive a survival benefit compared with the placebo (27). In recent years, numerous biomarkers, including genomic tumor markers, neoantigens, the tumor immune microenvironment phenotype, and liquid biopsy markers, have been evaluated in association with immune checkpoint inhibitors. Kumagai *et al* showed that in cancer patients, including GC, the frequency of PD-1+CD8+T cells relative to that of PD-1+ regulatory T cells in the tumor microenvironment predicts the clinical outcome of PD-1 blockade therapies and is superior to other biomarkers, including PD-L1 expression or tumor mutational burden (28). Recently, Kawakami *et al* demonstrated that baseline soluble PD-L1 levels in plasma were found to be a potential biomarker for predicting the efficacy of nivolumab in advanced GC (29). In addition, they showed that the combination of the Glasgow Prognostic Score (GPS) to soluble PD-L1, a prognostic indicator of inflammation and nutritional status, may be a more accurate predictive biomarker. However, such PD-L1 expression soluble PD-L1 are often challenging to measure and introduce in clinical practice. In contrast, the advantage of peripheral blood NLR is that it is routinely measured in cancer patients, so this parameter is readily available to physicians. Ogata *et al* reported that in 26 advanced GC patients treated with nivolumab, the median OS was significantly longer in GC patients with lower NLR values (30). NLR of patients treated with 3rd-line nivolumab may predict OS. Similarly, in our analysis of patients treated with 3rd-line nivolumab monotherapy, NLR before nivolumab induction could predict the prognosis of unresectable GC patients.

This study has some limitations. It is a single-center, retrospective study with a small sample size. Additionally, with concern to biomarkers, we did not examine or compare well-known prognostic biomarkers for GC patients, such as GPS (29), Prognostic Nutritional Index (31), or C-reactive protein/albumin ratio (32), to NLR. However, we identified associations between NLR and clinical outcomes in each chemotherapy line for the first time. Future prospective analyses will be required to confirm the results. In conclusion, NLR obtained before the initiation of each chemotherapy line might be an objective surrogate marker to predict prognosis for performance status.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Authors' contributions

HT performed all analyses, drafted the manuscript, and secured funding. MO, SY, and YY treated patients, collected clinical data and assisted with data interpretation. TN designed the project, assisted with interpreting all data, secured funding and drafted the manuscript. TY, KT and AN confirm the authenticity of all the raw data and performed statistical analyses. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the ethics committee of Kawasaki Medical School (IRB no.: 3939-01). The patients provided written informed consent, and all studies were conducted in accordance with The Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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