

**The pretreatment neutrophil-to-lymphocyte ratio predicts therapeutic response to radiation
therapy and concurrent chemo-radiation therapy in uterine cervical cancer**

(治療前の好中球/リンパ球比は子宮頸癌における放射線療法と化学療法同時放射線療法の
治療的反応性を予測する)

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Abstract

Objective: The aim of this study was to investigate the prognostic role of the pretreatment neutrophil-to-lymphocyte ratio (NLR) as a predictive marker prior to treatment of cervical cancer with radiation therapy (RT) alone or concurrent chemoradiation therapy (CCRT).

Methods: Fifty-six patients with squamous cell carcinoma (SCC) of the uterine cervix who underwent RT or CCRT from 2005 to 2013 at this Hospital were retrospectively identified using electronic databases. Patients were divided into a high NLR group (≥ 2.5) and a low NLR group (< 2.5). The efficacy of RT and CCRT in the two groups was compared.

Result: Of the 56 patients, 35 were in the high NLR group and 21 were in the low NLR group. In comparison to a high NLR, a low NLR was significantly associated with a complete response ($P < 0.001$). When cancer was divided into stages I/II and III/IV, patients with a low NLR had a significantly better therapeutic outcome than those with a high NLR ($P < 0.05$). Multivariate analysis showed that only the NLR was a significant prognostic factor for progression-free survival (PFS). Patients with a high NLR had a significantly shorter PFS and overall survival than those with a low NLR.

Conclusion: Results showed that a low NLR before treatment can predict a good response to RT or CCRT by all stages of uterine cervical cancer. The NLR may be a promising parameter on which to base the choice of a therapeutic strategy to treat SCC of the uterine cervix.

Keywords: NLR, uterine cervical cancer, radiation therapy, concurrent chemoradiation therapy, therapeutic outcome.

INTRODUCTION

Even though the incidence and mortality of invasive uterine cervical cancer have steadily decreased, this disease is still the second most common type of cancer in females worldwide and the leading cause of cancer deaths in women in developing countries [1].

The neutrophil-to-lymphocyte ratio (NLR) has been used as a cost-effective and simple parameter to indicate systemic inflammation or stress in critically ill patients without cancer [2]. The NLR may also relate to the prognosis of many types of cancer, including gastrointestinal tract malignancies [3], hepatocellular cancer [4], pancreatic cancer [5], non-small cell lung cancer [6], and urinary cancer [7]. Furthermore, the pretreatment NLR predicts survival in epithelial ovarian cancer [8] and uterine cervical cancer [9, 10]. However, the NLR was not a predictive factor in patients with uterine cervical cancer treated with neoadjuvant chemotherapy and radical hysterectomy [11]. Thus, the clinical implications of the NLR have yet to be determined.

Despite the improved survival rate for patients who receive concurrent chemo-radiation therapy (CCRT) [12], some patients are resistant to this treatment. The aim of this study was to investigate the prognostic role of the NLR as a pretreatment marker prior to treatment of uterine cervical cancer with radiation therapy (RT) alone or CCRT.

METHODS

Patients

Records of patients who had stage IB1 to IV uterine cervical cancer according to the staging system of the International Federation of Gynecology and Obstetrics (FIGO) were retrospectively reviewed using electronic databases. These patients were histologically diagnosed with squamous cell carcinoma and underwent RT or CCRT at Hirosaki University Hospital from 2005 to 2013. The following patients were excluded: 6 patients in whom the differential white blood count was not determined, 5 patients who had to be treated for another inflammatory disease just prior to RT or CCRT, and 1 patient who underwent rounds of RT at another hospital. This study ultimately examined the records of 56 patients.

Treatment

RT: External beam radiation was delivered with anteroposterior and posteroanterior opposed beams generated by an X-ray accelerator with an energy of 10 MV at a distance of 100 cm. Whole pelvis irradiation with a total dose of 50 Gy was delivered with a fraction dose of 2 Gy per day for 5 days a week over a 5-week period. After a dose of approximately 30 Gy, a central shield with a width of 4 cm at the midline was used and intracavitary brachytherapy was performed using a RALS with a Co 60 source. Intracavitary brachytherapy, with fractions of 6 Gy delivered to point A, was performed once a week for a total of 4 times [13].

Chemotherapy: Thirty mg/m² of nedaplatin was dissolved in a 500-mL saline solution and infused intravenously over 180 min. The first round was administered on the day that external beam RT started. The regimen was repeated weekly for a total of 6 times. Nedaplatin infusion was completed 1 hr before irradiation [13]. Nine patients who had not completed the concurrent chemotherapy schedule were also included in this study. Two patients used nedaplatin nor cisplatin, one used irinotecan, the other used paclitaxel and carboplatin.

Outcomes

The primary statistical end point was a tumor response, which was assessed by means of a clinical examination, including histological examination of the uterine cervix and a computed tomography scan from the chest to the pelvis. These examinations were performed about 1 month after completion of RT or CCRT. Patients with no evidence of disease were deemed to have a clinical complete response (CR). If tumors shrank after treatment but cancer cells or tumors were still noted, the patient was not deemed to have a CR.

Progression-free survival (PFS) was defined as the time between the date of the completion of treatment and the date of identification of progressive disease. We identified the case that tumor size were bigger than the size after treatment or new tumors were noted by a computed tomography scan or cancer

cells were noted by histological examination of the uterine cervix as progressive disease. Overall survival (OS) was defined as the time between the date of the completion of treatment and the date of death.

Calculation of NLR and determination of the NLR cut-off level

The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. Before treatment, the median NLR was 2.4, and patients were divided into a high NLR group (≥ 2.5) and a low NLR group (< 2.5).

Statistical analysis

The following variables were analyzed to predict tumor response and survival: FIGO stage, tumor size, treatment (patients were grouped as RT alone or CCRT), baseline hemoglobin (Hb) level, baseline white blood cell (WBC) and lymphocyte counts, and the NLR. The chi-square test and Student's t test were used to analyze categorical data, and the Welsh test was used for continuous variables. Receiver operating characteristic plots were conducted to determine the maximum sensitivity and specificity of a threshold value to stratify patients at high risk of recurrence. Relation of NLR to WBC, neutrophil and lymphocyte was calculated using Spearman's rank correlations coefficient.

Multiple logistic regression models were used to identify independent prognostic factors for a response and PFS. The following factors were included in the multivariable models: age, FIGO stage, pretreatment SCC value, tumor size, baseline neutrophil and lymphocyte counts, Hb level, and the NLR.

The OS and PFS were calculated using the Kaplan-Meier method. A log-rank test was used to assess statistical significance. All p values were two-tailed and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS (version 21, SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

This study analyzed data from 56 patients with a median age of 65.1 years (range, 35-89 years). In terms of the FIGO stage, 13 patients had stage I cancer, 11 had stage II, 17 had stage III, and 15 had stage IV. Of these 56 patients, 35 were in the high NLR group and 21 were in the low NLR group. All pretreatment NLR values according to stage are plotted in Fig.1. Clinical characteristics of patients and tumors by the pretreatment NLR are shown in Table 1. As seen in this Table, patients with ≥ 2.5 had advanced tumor in terms of tumor size, SCC level and lymph node metastasis.

When this analysis was performed, clinical outcomes were recorded for 47 patients (83.9%) and 2 patients (3.6%) who had been followed until recurrence. Cancer recurred in 20 patients (35.7%) and 16 patients (28.6%) died due to the cancer. The mean period to recurrence was 5.3 months (range: 1 to 18 months) and the mean period to disease-related death was 14.2 months (range: 2 to 41 months).

Pretreatment factors and therapeutic response

At the completion of treatment, 25 patients (44.6%) had a clinical CR. Table 2 shows relationship between pretreatment factors and complete response obtained from our subjects. As shown in Table 2, univariate logistic regression analysis found a significant association between CR and FIGO stage, tumor size, type of treatment, WBC count, neutrophil count and NLR. In this study, a higher baseline neutrophil count was also a significant predictor of non-response (Table 2). In comparison to a high NLR, a low NLR was significantly associated with a CR ($P < 0.00005$) (Table 3). When cancer was divided into stages I/II and III/IV, patients with a low NLR had a significantly better therapeutic outcome than those with a high NLR (Table 3).

In multivariate logistic regression analysis with regard to CR, variables that were considered to statistically significant factors in univariate regression were selected. These variables were the FIGO stage, tumor size, treatment, and the NLR. The pretreatment NLR was closely correlated with the pretreatment WBC count and neutrophil count, as indicated by Spearman's rank correlation coefficients (Table 4). From among the variables WBC count, neutrophil count, and the NLR, only the NLR was selected as the most significant factor ($p=0.0015$) because of multi-collinearity. Multivariate logistic regression revealed that the NLR was a significant prognostic factor with an odds ratio of 0.35 (95% confidence interval; 0.17-0.74, Table 5). Receiver operating characteristic (ROC) curves were derived for age, SCC, tumor size, the NLR, and Hb levels with CR as an explanatory variable. Examination of the parameters that had the largest positive likelihood ratios indicated that a cut-off level of 2.5 for the NLR had the largest positive likelihood ratio. This level was close to the median NLR (Table 6).

The relationship between pretreatment factors and PFS and OS

Univariate and multivariate analyses revealed that only the NLR was a significant prognostic factor for PFS (Table 7 and 8). On the other hand, NLR and treatment were associated with OS in univariate analyses, and in multivariate only lower NLR was associated with longer survival (Table 9 and 10). Patients with a high NLR had a significantly shorter PFS and OS than those with a low NLR (Fig. 2A and B). The pretreatment hemoglobin level was closely associated with PFS ($p=0.058$) (Table 8).

DISCUSSION

Previous studies [14, 15] have shown that FIGO stage, Hb level, and tumor size are significant prognostic factors of cervical cancer and the results of the present study were compatible as shown in Table 1. In addition, treatment modality (CCRT vs. RT) was also reported to be an impact prognostic factor, as CCRT had a higher rate of success than RT alone [12]. The present study showed that the NLR before treatment was significantly related to the outcomes of radiation for the uterine cervical cancer, and supported results shown by Lee et al. [9] and Zhang et al. [10]. In addition, the present study showed that a low NLR (less than 2.5) was significantly associated with a CR and longer PFS and longer OS in comparison to a high NLR (2.5 and over).

The fact that a high NLR is a factor for a poor prognosis after surgery or after chemotherapy has been reported in relation to various types of carcinomas [3-10]. Looking specifically at cervical cancer, Lee et al. reported that the NLR may be a predictor of prognosis for RT after surgery or definitive RT [9]. The current study involved a choice of only RT or CCRT to treat cervical cancer, but the results were similar to those of Lee et al.

An increase in neutrophils or a decrease in lymphocytes is reported to be associated with a shorter survival time. Why does a high NLR diminish the effectiveness of therapy and why does it increase recurrence and predict a poor prognosis? The specific mechanisms by which it causes these phenomena have yet to be identified. A G-CSF-producing tumor is typically characterized by a rapid progression and obvious leukocytosis, resulting in a poor prognosis. In 4 patients with G-CSF-producing cervical cancer, Matsumoto et al. reported that the cancer recurred in all 4 patients within 6 months and that all of the patients died within 15 months [16]. In addition, the presence of inflammatory foci may increase the risk of cancer progression. Inflammation is known to change a tumor's microenvironment and promote angiogenesis and metastasis [17]. Inflammatory changes are normally accompanied by an increase in neutrophils. Therefore, one could logically predict that cervical cancer and associated leukocytosis would have a poor prognosis. Recently, a view on the reason for the poor prognosis of cervical cancer and associated leukocytosis has been presented. G-CSF is produced by a tumor and in turn induces the production of myeloid-derived suppressor cells (MDSCs). These MDSCs cause rapid progression of cervical cancer and leukocytosis and radioresistance [18]. Mabuchi et al. depleted MDSCs, which succeeded in inhibiting the progression of cervical cancer and leukocytosis and enhancing radiosensitivity [18]. MDSCs have been found in bone marrow, blood, and the spleen of individuals with tumors. In response to the progression of inflammation and cancer, these cells spread throughout the body as part of the host's immune response [19]. Although cisplatin is a key drug in CCRT to treat cervical cancer, a study has reported that it induces MDSCs [20]. Meanwhile, gemcitabine is known to be an anticancer drug that reduces the number of MDSCs [21], then gemcitabine may be favorable for chemoradiation therapy of cervical cancer patients with high NLR.

Recent studies have found increased levels of pro-inflammatory cytokines in patients with an increased NLR [22, 23] and increased peri-tumor macrophage infiltration [24]. These results suggest that an increased NLR reflects an up-regulated innate immune response [21]. The immune response has been found to differ depending on the type of cancer. With pancreatic cancer, a marked increase in lymphocytes occurs preoperatively and postoperative [25]. The NLR was not associated with the prognosis of pancreatic cancer. In addition, a study has reported that combining chemotherapy and polysaccharide K (PSK) after surgery for colon cancer allows the NLR (which is correlated with prognosis) to be kept to a low level, improving the patient's prognosis [26]. Chemotherapy triggers the apoptosis of peripheral blood T lymphocytes, but immunotherapy with PSK is presumably effective because it controls that apoptosis [27].

RT is known to primarily have little effect in a hypoxic state. A hypoxia state is related to inflammation and anemia, and elements of the innate inflammatory response are associated with the up-regulation of growth factors and angiogenesis [28, 29]. Systemic inflammatory responses are associated with alteration in the circulating WBC count, with neutrophilia and relative lymphopenia [30]. Santin et al. investigated lymphocyte populations including CD3+, CD4+ and CD8+ T-cell subsets, B cells (CD19+), and natural killer cells (CD56+, CD16+, CD3-) before, during, and after RT in patients with locally advanced cervical cancer [31]. While both RT and CCRT with cisplatin significantly decreased the mean absolute number of all lymphocyte subsets compared to pretreatment levels, Santin et al. detected no differences in the characteristics or the magnitude of the lymphopenia induced by the two treatments. Thus, cisplatin does not enhance the magnitude or characteristics of radiation-induced immunosuppression [31].

In conclusion, the current study showed that a low NLR before treatment can predict a good response to RT or CCRT in terms of CT and PFS by all stages of uterine cervical cancer. The NLR may be a promising parameter on which to base the choice of a therapeutic strategy to treat SCC of the uterine cervix. This study involved a relatively small sample size, so further validation is required prior to incorporating the NLR as a prognostic indicator.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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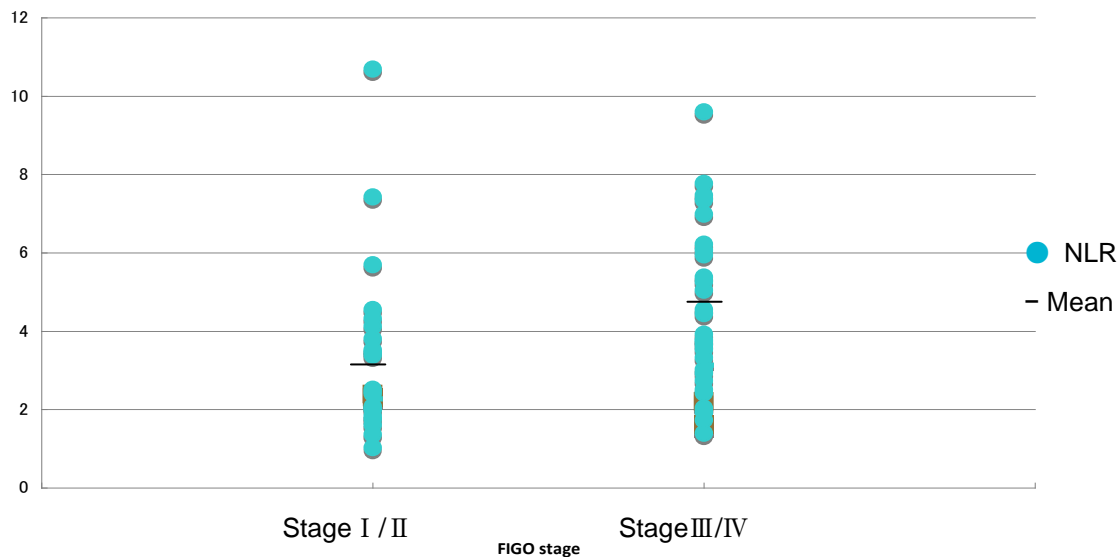
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Figure legend

Fig. 2. A, Kaplan-Meier survival curves for progression-free survival (PFS) of patients with a high NLR and those with a low NLR. Patients with a high NLR had a significantly shorter PFS than those with a low NLR (log-rank test, $P < 0.05$). B, Kaplan-Meier survival curves for overall survival (OS) of patients with a high NLR and those with a low NLR. Patients with a high NLR had a significantly shorter OS than those with a low NLR (log-rank test, $P < 0.001$).

Figure1.



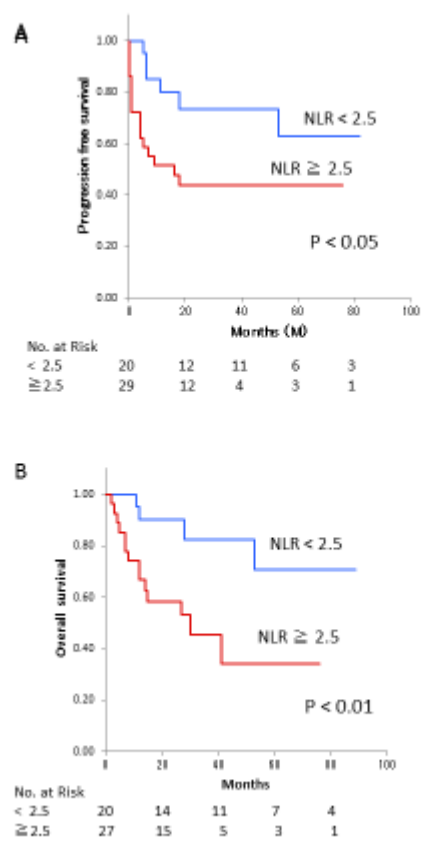


Figure 2

Table 1. The relationship between NLR status and clinical factors (n=56)

Clinical factors	NLR < 2.5 (n=21)	NLR ≥ 2.5 (n=35)	P value
Mean age (y)	68.8 ± 2.7	62.8 ± 2.4	0.12*
FIGO stage			
Stage I/ II	13 (62%)	11 (31%)	< 0.05**
Stage III/IV	8 (38%)	24 (69%)	
Mean SCC value (ng/ml)	10.2 ± 2.6	41.1 ± 13.2	< 0.001*
Tumor size			
<5cm	16 (76%)	14 (41%)	< 0.05**
≥5cm	5 (24%)	20 (59%)	
Lymph node metastasis			
Yes	3 (14%)	18 (51%)	< 0.005**
No	18 (86%)	17 (49%)	
Treatment			
RT alone	10	9	0.09**
CCRT	11	26	

*Student's t-test

**Chi-square test

Table 2. The relationship between pretreatment factors and complete response

Variables		Odds ratio	95% Confidence interval	P value*
Age (y)	10 age increments	1.03	0.99 - 1.08	0.15
FIGO stage	I/ II vs. III/IV	0.29	0.09-0.95	0.04
Tumor size (cm)	<5 vs. ≥ 5	0.67	0.48 - 0.91	0.01
SCC value (ng/ml)	1ng/ml increments	0.99	0.98 - 1.0	0.31
Treatment	RT vs. CCRT	0.21	0.05 - 0.81	0.02
WBC ($\times 10^9/L$)	$1 \times 10^9/L$ increments	0.43	0.25 – 0.74	0.0023
Neutrophil ($\times 10^9/L$)	$1 \times 10^9/L$ increments	0.19	0.07 – 0.57	0.0031
Lymphocyte ($\times 10^9/L$)	$1 \times 10^9/L$ increments	0.93	0.32 – 2.73	0.90
NLR	1 increments	0.33	0.17 - 0.66	0.0015
Hb (g/dL)	1g/dl increments	1.25	0.94 - 1.65	0.13

*Univariate logistic regression analysis with regard to complete response

Table 3. NLR value and therapeutic outcome

		No. of patients	Complete response		P value*
			Cases	%	
NLR					
	<2.5	21	17	81.0	<0.00005
	≥2.5	35	8	22.9	
FIGO stage I/ II					
	NLR <2.5	13	11	84.6	<0.05
	NLR ≥2.5	11	4	36.3	
FIGO stage III/IV					
	NLR<2.5	8	6	75.0	<0.005
	NLR ≥2.5	24	4	16.7	

* Chi-square test

Table 4 The correlation of NLR with WBC, neutrophil and lymphocyte

Variables		ρ	P value*
NLR	WBC	0.65	0.0000
NLR	Neutrophil	0.79	0.0000
NLR	Lymphocyte	-0.40	0.005

*Spearman's rank correlation coefficient

Table.5 Multiple logistic regression analysis with regard to complete response

variables		Odds ratio (95% CI)	P value
FIGO stage	Stage I/ II	1 (referent)	0.52
	Stage III/IV	0.56 (0.10-3.23)	
Tumor size	1cm increments	1.03 (0.67 - 1.56)	0.91
Treatment	RT	1 (referent)	0.39
	CCRT	0.41 (0.05 - 3.17)	
NLR	1 increments	0.35 (0.17 - 0.74)	0.006

CI: confidence interval

Table 6 Receiver operating characteristic (ROC) analysis

Variables	Odds ratio	Cut-off value
Age (y)	0.89	53
SCC (ng/ml)	4.29	4.1
Tumor size (cm)	2.86	5.5
NLR	25.9	2.52
Hb (g/dl)	0.75	11.1

Table 7 Univariate analysis for progression free survival (PFS)

variables		Median PFS (M)	P value*
Age (y)	<65	38.4	0.32
	≥ 65	51.8	
FIGO stage	Stage I/ II	56.0	0.16
	Stage III/IV	38.7	
SCC (ng/ml)	<20	47.0	0.66
	≥ 20	42.6	
Tumor size (cm)	<5	51.5	0.40
	≥ 5	39.7	
Treatment	RT	45.7	0.19
	CCRT	42.0	
Hb (g/dl)	<11	37.5	0.12
	≥ 11	50.3	
NLR	<2.5	59.7	0.04
	≥ 2.5	35.9	

* logrank test

Table 8 Multivariate analysis for progression free survival

Variables		Cox proportional hazard model	
		HR (95% CI)	P value
FIGO stage	Stage I/ II	1 (referent)	0.25
	Stage III/IV	1.91 (0.75-4.75)	
Tumor size (cm)	< 5	1 (referent)	0.16
	≥ 5	0.82 (0.61-1.09)	
Treatment	RT	1 (referent)	0.96
	CCRT	0.97 (0.24-3.88)	
Hemoglobin (g/dl)	<11	1 (referent)	0.058
	≥ 11	0.82 (0.67-1.01)	
NLR	< 2.5	1 (referent)	0.001
	≥ 2.5	1.53 (1.19-1.97)	

HR: hazard ratio, CI: confidence interval

Table 9 Univariate analysis for overall survival (OS)

variables		Median OS (M)	P value*
Age (y)	<65	40.9	0.11
	≥ 65	66.4	
FIGO stage	Stage I/ II	66.8	0.056
	Stage III/IV	45.6	
SCC (ng/ml)	<20	56.3	0.69
	≥ 20	53.7	
Tumor size (cm)	<5	62.4	0.12
	≥ 5	42.5	
Treatment	RT	58.7	<0.05
	CCRT	47.8	
Hb (g/dl)	<11	42.4	0.12
	≥ 11	62.9	
NLR	<2.5	72.4	<0.01
	≥ 2.5	37.7	

* logrank test

Table 10 Multivariate analysis for overall survival

Variables		Cox proportional hazard model	
		HR (95% CI)	P value
FIGO stage	Stage I/ II	1 (referent)	0.23
	Stage III/IV	2.17 (0.61-7.70)	
Tumor size (cm)	< 5	1 (referent)	0.22
	≥ 5	1.86 (0.68-5.09)	
Treatment	RT	1 (referent)	0.28
	CCRT	3.23 (0.38-27.41)	
Hemoglobin (g/dl)	<11	1 (referent)	0.24
	≥ 11	0.54 (0.19-1.52)	
NLR	< 2.5	1 (referent)	<0.01
	≥ 2.5	2.80 (0.83-9.34)	

HR: hazard ratio, CI: confidence interval

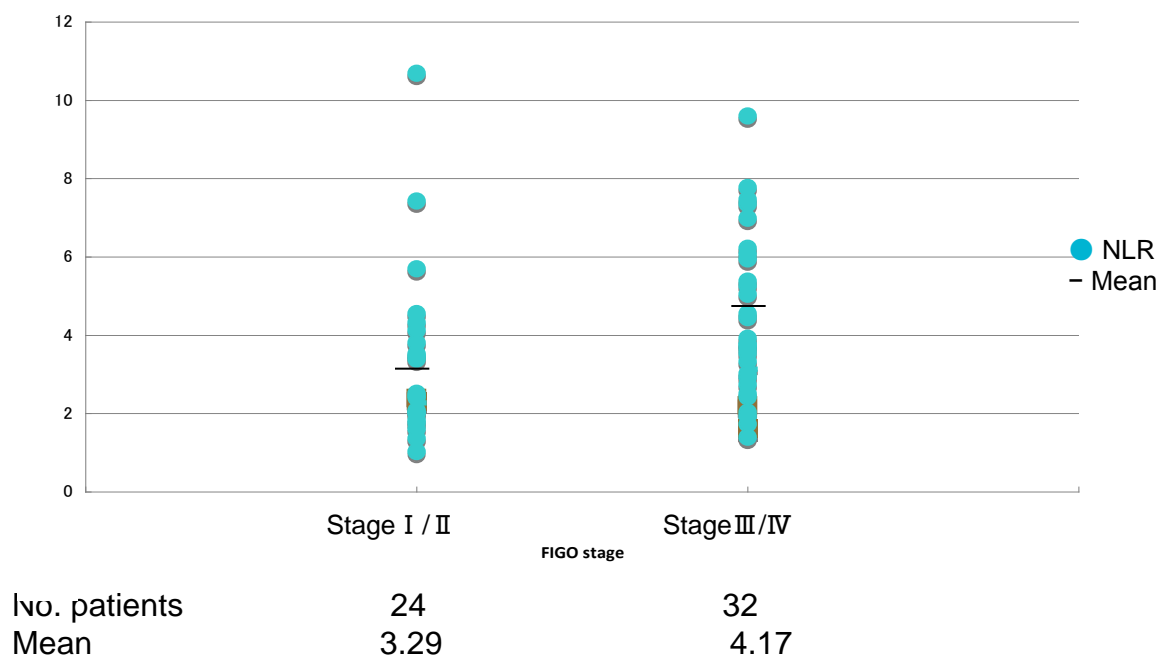


Figure 1

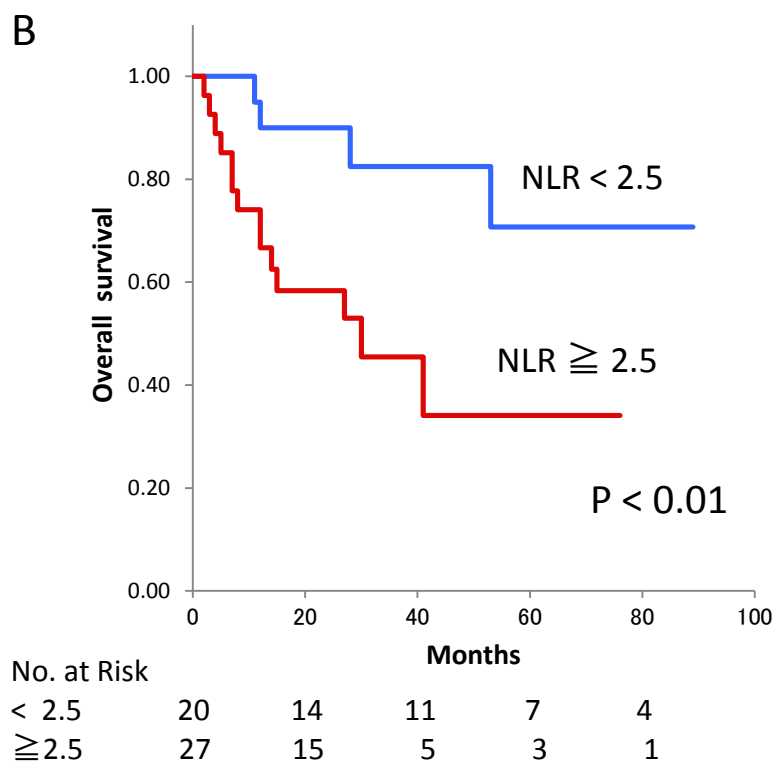
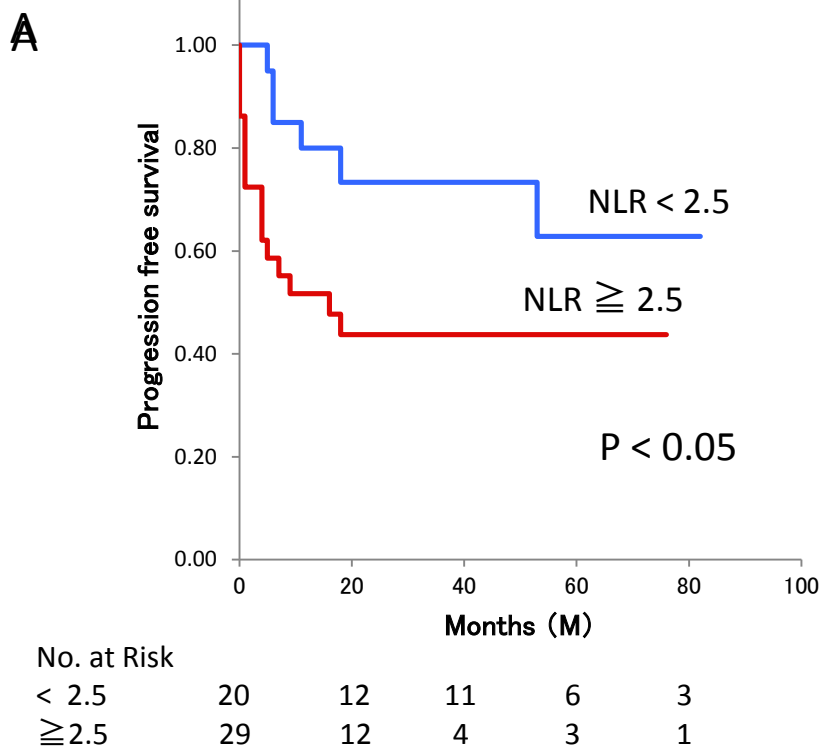


Figure 2