

The Impact of Radiotherapy Parameters on Lymphopenia and Overall Survival in Patients With Esophageal Cancer

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Research

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Abstract

Purpose

We sought to perform survival analysis of patients with thoracic esophageal squamous cell carcinoma (ESCC) receiving definitive radiotherapy and identify prognostic factors among hematological and dosimetric factors.

Methods

Cases of thoracic ESCC treated with chemoradiation between 2014 and 2017 were identified. The impact of clinicopathological factors' on overall survival (OS) was analyzed via Cox proportional hazards model. Absolute lymphocyte counts (ALC) and the neutrophil-to-lymphocyte ratio (NLR = ANC/ALC) were assessed before radiotherapy (RT), during RT, and after RT. Cox regression was used to correlate clinical factors with both hematologic toxicities and overall survival. Multiple logistic regression analyses were used to find associations between lymphopenia and dosimetric parameters. The receiver operating characteristics (ROC) curve was used to determine cut-off points.

Results

Ninety-nine ESCC patients were enrolled with the median overall survival of 23 months. The median RT dose was 55.75Gy(46–66Gy), and the mean does (D_{mean}) of thoracic vertebrae dose (TVB) was 27.04 ± 9.65 Gy. Based on multivariate analysis, V20 of TVB, pretreatment NLR, and ALC nadir were associated with a worse OS significantly. Concurrent CRT, increasing mean TVB dose and V20 of TVB were associated with higher odds of lymphopenia risk ($P \leq 0.05$) through multiple logistic regression analysis.

Conclusions

In ESCC patients who received definitive RT, V_{20} of TVB, pretreatment NLR, and ALC nadir during RT were independent prognostic factors and chemotherapy regimen, mean TVB dose, and V_{20} of TVB were associated with lymphopenia.

Introduction

Esophageal cancer (EC) is an often-fatal type of malignancy in our country with the incidence of morbidity and mortality ranking sixth and fourth, respectively ⁽¹⁾. In China, ESCC is the leading histology subtype and most patients have developed to be in the middle or advanced stage, whereby a surgical operation is not applicable at the time of initial diagnosis(2). CRT or radiation is the definitive non-surgical approach of non-metastatic esophageal cancer. More recently, with the increasing appreciation, immunotherapy has brought a new paradigm in cancer therapy and demonstrated significant clinical

benefits⁽³⁾. There is more evidence of the relationship between radiotherapy and the immune function of lung cancer, but little about esophageal cancer.⁽⁴⁾

Immune and inflammatory responses are the vital processes of tumor progression⁽⁵⁾. Radiation can suppress host immunity by killing immune cells, in particular cytotoxic T lymphocytes⁽⁶⁾. The radiation-induced lymphopenia (RIL) is a common hematologic adverse effect during RT as peripheral lymphocytes are known to be the most radiosensitive cells. In the RT procedure, RIL in the tumor microenvironment may promote tumor progression and ALC nadir was proved to be correlated with poor survival in a wide variety of malignancies, such as glioblastoma, cervical cancer, pancreatic cancer, and non-small cell lung cancer (NSCLC)⁽⁷⁻¹⁰⁾. The NLR, an index of generalized inflammatory response has also been demonstrated as a prognostic factor for patients with multiple cancer types⁽¹¹⁾.

However, to the best of our knowledge, relevant studies revealing immunosuppression and overall outcomes in ESCC are limited. The purpose of this study was to identify the effect of hematological toxicity and radiation parameters on the overall survival of ESCC patients, as well as exploring the relationship between lymphopenia and radiation parameters.

Materials And Methods

Patient selection and data collection

We retrospectively reviewed the medical records of 318 consecutive patients with esophageal cancer who were treated at the Second Affiliated Hospital of Anhui Medical University between February 2014 and November 2017. The specific inclusion criteria were: (1) patients with stage I-IV_A esophageal carcinoma (according to the 8th Union for International Cancer Control (UICC) esophageal cancer staging⁽¹²⁾); (2) histological pathologic confirmation being limited to squamous cell carcinoma; (3) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 ; (4) the primary site of esophageal carcinoma being limited to thoracic segments; (5) patients who completed definitive radiotherapy (with or without chemotherapy) of intensity modulated radiation therapy (IMRT) no less than 45Gy without delay; and (6) having complete blood counts (CBC) and retrievable dosimetry records (heart, lung, thoracic vertebrae and whole body dose).

The exclusion criteria were: (1) patients who received surgery; (2) the patients' follow-up being less than one month or unknown; and (3) the necessary and sufficient follow-up data included documented CBC values either before, during, and/or after RT, retrievable and complete DVH information was lacking.

Radiation treatment and dosimetric analysis

Radiotherapy was performed using a varian linear accelerator. All 99 esophageal cancer patients received IMRT. Our current practice contoured gross tumor volume (GTV) according to chest positioning CT, esophageal barium meal imaging and electronic gastroscopy, encompassing the primary tumor and positive lymph nodes if present. For upper thoracic cancer, clinical tumor volume (CTV) involved bilateral

supraclavicular and upper mediastinal lymph nodes areas. For middle and lower thoracic cancer, CTV involved abdominal and middle and lower mediastinal lymph nodes. CTV was expanded from GTV with 3-4cm superiorly and inferiorly and 0.8cm radially. Planning tumor volume (PTV) was defined by a 0.5- to 1-cm expansion from the CTV.

Chemotherapy

The chemotherapeutic regimen consisted of intravenous infusional Cisplatin (25 mg/m²) combined with Fluorouracil (300 mg/ m²; continuous infusion) or Paclitaxel (45 mg/m²). Chemotherapy was performed and repeated every week. Chemotherapy was held or reduced in dose when grade ≥2 hematologic toxicity occurred.

Data collection

Clinicopathologic variables including age, gender, location, performance status, stage, use of concurrent or sequential chemotherapy, and radiation dosimetry parameters were obtained. From CBC data, ALC and absolute neutrophil counts (ANC) were recorded within two weeks before RT start. The nadir of ALC was the lowest ALC appearing within two months after the RT start. The NLR, calculated by dividing the ANC by the ALC.

The following radiotherapy-related variables were assessed from the DVH parameter: mean heart dose (MHD), mean lung dose (MLD), mean body dose (MBD), mean vertebral dose (MVD) and V5-20 of TVB from T1-T12. The estimated dose of radiation to immune cells (EDRIC) compromising MHD, MLD, MBD, and number of fractions as a model developed by Jin et al, confirmed a correlation with OS in NSCLC⁽¹³⁾. We take EDRIC into this study with the model as follows:

$$\text{EDRIC} = 0.12 \cdot \text{MLD} + 0.08 \cdot \text{MHD} + [0.45 + 0.35 \cdot 0.85 \cdot (\text{of fractions}/45)^{1/2}] \cdot \text{MBD}$$

Statistical analysis

The primary endpoint was OS, defined as between the date of starting radiotherapy and the death or final follow-up(censored). The continuous baseline clinicopathological characteristics were analyzed using descriptive statistics, and categorical data were tabulated as frequencies and percentages.

The Kaplan–Meier (KM) was performed to estimate survival curves, and the log-rank test was used to compare survival curves. The potential prognostic factors were explored using Cox regression and reported as hazard ratios (HR) and 95% confidence interval. Due to the hematologic index and dosimetric parameters being continuous data, ROC curves were used to determine the cut-off value of the best predictors of overall survival. All tests were two-sided, and *P*-values less than 0.05 were considered significant.

Statistical analyses were performed with SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and the survival curves were plotted by GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA)

Results

Overall, 99 esophageal squamous cell carcinoma patients were enrolled. Of these patients, 79 were male and 20 were female. The median age of all patients included in the study was 67 years (range, 43–83). The majority of cases were located in the middle segment of the esophagus (55%), followed by lower esophageal cancer (25%), and only 20 cases in the upper segment. There were 55 cases in Stage III, 41 cases in Stage II and 3 cases in Stage IA. The median follow-up of enrolled patients was 24.69 months (range 4–73 months). Most patients received concurrent chemoradiotherapy (59%), and nine (9%) of them were treated with sequential chemoradiotherapy. Thirty-two (32%) patients were treated with RT alone due to the toxicity of chemotherapy or self-refusal. The prescribed dose was 46–66 Gy/1.8-2.2Gy per fraction with a median dose of 55.75Gy(46–66Gy), and the D_{mean} of TVB was $27.04 \pm 9.65\text{Gy}$. (Tab.1)

Overall survival

In the entire set of patients, the estimated median overall survival was 23 months (95%CI: 18.45-27.56), and three- and five- year OS rates were 30% and 18%, respectively.

ROC curve analysis showed that the cut-off values of pretreatment NLR and ALC nadir were 2.0 and $0.3 \times 10^9/\text{L}$ and the value of D_{mean} of TVB was 28.94Gy, of V5, v10, and V20 of TVB it was 72%, 68.1%, and 80%, respectively. The cut-off value of EDRIC was 7.11Gy.

Factors correlated with OS were summarized in Table 2. Based on univariate analysis, increased D_{mean} and V_{20} of TVB, EDRIC were associated with poorer OS. Stage (HR, 2.051; 95% CI, 1.236-3.405; $P = 0.003$), pretreatment NLR (HR, 2.062; 95% CI, 1.278-3.326; $P = 0.003$), ALC nadir (HR, 0.542; 95% CI, 0.317-0.929; $P = 0.026$), V_{20} of TVB (HR, 2.888; 95% CI, 1.450-5.753; $P = 0.003$) were significantly associated with overall outcomes by multivariate analysis.(Fig. 1).

Multiple logistic regression analysis of lymphopenia

In order to better understand the impact of the radiation dose of thoracic vertebrae and lymphopenia, further statistical analysis was performed. Multiple logistic regression analyses were summarized in Table 3, the chemotherapy regimen, EDRIC, D_{mean} and V_{20} of TVB remained correlated with the increase in lymphopenia odds statistically significantly ($P < 0.05$).

Discussion

In this study, we found stage, pretreatment NLR, ALC nadir, and V_{20} of TVB to be independent predictors of survival outcomes for the locally advanced ESCC patients who underwent definitive chemoradiotherapy.

As the marker of inflammation and immunosuppression, pretreatment NLR and ALC nadir during treatment have been determined as prognostic factors in various solid tumors, in particular lung

cancer(14-16).

In 2014, Tang et al. reported a dataset of 711 NSCLC patients who were treated with definitive radiotherapy and found lower lymphocytes nadirs and larger GTVs predicting worse outcomes⁽¹⁰⁾.

Ryoko Suzuki *et al*(14) retrospectively reviewed the clinicopathologic and treatment characteristics of 252 patients with ES-SCLC. Multivariate analysis identified low TLC and high NLR before the treatment as predicting inferior survival. In recent preclinical study, the grade 4 lymphopenia seemed to predict the worse progression-free and overall survival in esophageal cancer cohort⁽¹⁷⁾. In this study, we demonstrated that pretreatment NLR and ALC nadir during definitive RT were significantly associated with worse outcomes.

How does radiation give rise to lymphopenia? Previous preclinical and clinical studies have revealed that the radiation interacting with the host immune system through activating innate and adaptive antitumor immune responses^(18, 19). However, the mechanism of immunosuppression contributing to radiation-induced lymphopenia remains unresolved. It may seem due to the greater lymphocyte's exposure of disease sites and larger radiation portals.

The radiation of thoracic malignancies are often close to the heart, such as esophageal, lung and left-sided breast cancers which are encompassed in the radiation portal. Therefore, the increasing dose of heart doses, lung and esophageal would result in strong lymphopenia^(6, 10, 20). RTOG 0617, a randomized phase III clinical trial, has also revealed the potential for radiation acts as a relevant factor reducing immune function. From a survey of patients who received concurrent CRT of locally advanced NSCLC, heart V5 and heart V30 were indicated as being predictors of outcomes. On multivariate analysis, the higher cardiac dose was related to poorer survival(21).

Another retrospective study of 117 patients who underwent definitive treatment for stage III NSCLC found that the EDRIC was the independent prognosticator of outcomes⁽¹³⁾. We take the model of EDRIC for reference but did not obtain the same conclusions. In our study, EDRIC shows the statistically significant differences in univariate analysis only, not in the multivariate Cox regression. We suppose that there are two possible reasons: (1)when variables of TVB and EDRIC entered into Cox regression simultaneously, an interaction existed between them, which interferes with the outcome; (2) the relative contribution of EDRIC to outcome is likely to be relatively small compared with that of TVB in ESCC.

In addition to the above standpoint, the unintentional RT to lymph node basins and secondary lymphoid organs like bone marrow, thymus and other potential organs may cause lymphopenia, as these sites and the tumor itself are the key organs induced by a direct hit of lymphocytes by RT. The bone marrow, pelvis, cervical vertebrae and thoracic vertebrae are the top three sites of hematopoiesis by activating the proliferating bone marrow⁽¹⁶⁾. Therefore, RT doses to pelvic, cervical, and thoracic vertebrae are potential drivers of BM suppression.

Several studies have confirmed that RT dose contributes to BM suppression in the pelvis(22, 23). Recently, a study of 201 patients with NSCLC and SCLC received definitive chemoradiation demonstrated increasing mean TVB dose and V5-V20 of TVB were correlated with higher odds of grade ≥ 3 hematologic toxicities(13). However, only logistic analyses were performed to explore the correlation of TVB dose with HT3+ in this study without direct evidence of TVB dose and the overall survival of patients who received CRT.

We assumed in the procedure of chemoradiotherapy that thoracic vertebrae dose correlates with ALC nadir due to immunosuppression. In this study, multiple regression analysis confirmed this hypothesis(V20 of TVB $< 80\%$ reduced the risk of ALC nadir $\times 0.3 \times 10^9/L$), and multivariate COX analyses have shown that V20 of TVB is an independent predictor for ESCC patients in our cohort. To my knowledge, this is the first study between vertebral dose and prognosis in esophageal cancer.

The present study did have several limitations. First, the retrospective study enrolled from a single center in China, so there exists a risk of bias in selection and information. Second, our patient cohort comprised only 99 patients, and we counted several radiation parameters including the D_{mean} and V5-50 of heart and D_{mean} and V5-30 of lungs but did not enter them into the Cox regression due to the small patient cohort. Finally, identifying the cut-off values of categorical data by using ROC curve analysis may not provide the most accurate results.

Thus, a larger, multi-institutional study is necessary to verify our results.

In conclusion, our study demonstrated that D_{mean} and V20 of TVB might be clinically useful to help to predict severe lymphopenia of patients of locally advanced ECSS receiving definitive radiotherapy. Increased V20 of TVB, as well as pretreatment NLR and decreased ALC nadir were associated with poorer clinical outcomes. D_{mean} of TVB below 28.49Gy and TVB V20 $\leq 80\%$ are correlated with lower ALC nadir. Optimizing prescription or treatment planning approaches to minimize mean dose and V20 of TVB may improve outcomes.

Abbreviations

ESCC: Esophageal squamous cell carcinoma

OS: Overall survival

ALC: Absolute lymphocyte counts

ANC: Absolute neutrophil counts

NLR: Neutrophil-to-lymphocyte ratio

RT: Radiotherapy

ROC: Receiver operating characteristics

D_{mean}: Mean dose

TVB: Thoracic vertebrae dose

CRT: Chemoradiation therapy

EC: Esophageal cancer

RIL: Radiation-induced lymphopenia

NSCLC: Non-small cell lung cancer

UICC: Union for International Cancer Control

ECOG: Eastern Cooperative Oncology Group

IMRT: Intensity modulated radiation therapy

CBC: Complete blood counts

GTV: Gross tumor volume

CTV: Clinical tumor volume

PTV: Planning tumor volume

MHD: Mean heart dose

MLD: Mean lung dose

MBD: Mean body dose

MVD: Mean vertebral dose

EDRIC: Estimated dose of radiation to immune cells

HR: Hazard ratios

Declarations

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Contributions

Ming Liu wrote the manuscript. All authors read and approved submission of the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Anhui Medical University (approval No. 20180033).

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no conflict of interest.

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Tables

Table 1 Clinicopathological characteristics

Characteristic	Median (Range or %)
Gender	
Male	79(80%)
Female	20(20%)
Age, years	67(43–83)
ECOG Score	
0-1	69(70%)
2	30(30%)
Tumor location	
Upper	20(20%)
Middle	54(55%)
Lower	25(25%)
Clinical stage	
I	0
II	55(56%)
III	41(41%)
IV _A	3(3%)
Chemotherapy regimen	
CRT	58(59%)
Sequential RT	9(9%)
RT alone	32(32%)
Prescribed RT dose[Gy]	55.75(46–66)
<6000	51(51%)
≥6000	48(48%)
Dose[Gy]/fraction	1.8–2.2

Table 2 Cox regression of clinical and dosimetric variables with overall survival

Characteristic	Univariate	Multivariate
	HR (95%CI), <i>P</i> value	HR (95%CI), <i>P</i> value
Gender (male vs. female)	NS	NS
Age, years		NS
≤65 (ref.)	NS	
>65		
ECOG Score		
0-1(ref.)	NS	NS
≥2		
Tumor location		
Upper	Ref	Ref
Middle	NS	NS
Lower	NS	NS
Chemotherapy regimen		
CRT	Ref	Ref
Sequential RT	NS	NS
RT alone	NS	NS
Clinical stage		
II~III (ref.)	1.893(1.188-3.017)	2.051(1.236-3.405)
IV~V	<i>p</i> =0.007	<i>p</i> =0.003
Prescribed RT dose (Gy)		
<60 (ref.)	NS	NS
≥60		
Pretreatment NLR		
<2(ref.)	2.216(1.379-3.562)	2.062(1.278-3.326)
≥2	<i>p</i> =0.001	<i>p</i> =0.003
ALC Nadir (*10 ⁹ /L)		
<0.3(ref.)	0.596(0.365-0.971)	0.542(0.317-0.929)
	<i>p</i> =0.036	<i>p</i> =0.026

≥ 0.3		
D _{mean} (Gy)		
<28.94(ref.)	1.623(1.021-2.580)	NS
≥ 28.94	<i>p</i> =0.041	
V5 (%)		
<71.2(ref.)	NS	NS
≥ 71.2		
V10 (%)		
<68.9(ref.)	NS	NS
≥ 68.9		
V20 (%)		
<80(ref.)	2.911(1.47-5.767)	2.888(1.450-5.753)
≥ 80	<i>p</i> =0.002	<i>p</i> =0.003
EDRIC (Gy)		
<7.11(ref.)	1.91(1.089-3.35)	NS
≥ 7.11	<i>p</i> =0.024	

Table3 Multivariate logistic regression of the risk factors related to ALC nadir

Variables	Multiple regression	
	OR (95%CI)	<i>P</i> value
Prescribed RT dose (Gy)		
<60	Ref	
≥60	1.935(0.659-5.683)	<i>p</i> =0.230
Chemotherapy regimen		
RT alone	Ref	
CRT	4.764 (1.555-14.590)	<i>p</i> =0.019
Sequential RT	1.026(0.129-8.147)	<i>p</i> =0.981
D _{mean} (Gy)	6.822(1.090-42.706)	<i>p</i> =0.04
V5(%)	1.333(0.364-4.878)	<i>p</i> =0.664
V10(%)	1.447(0.084-2.383)	<i>p</i> =0.346
V20(%)	1.591 (1.336 -1.894)	<i>p</i> =0.046
EDRIC (Gy)	0.209(0.057-0.762)	<i>p</i> =0.018

Figures

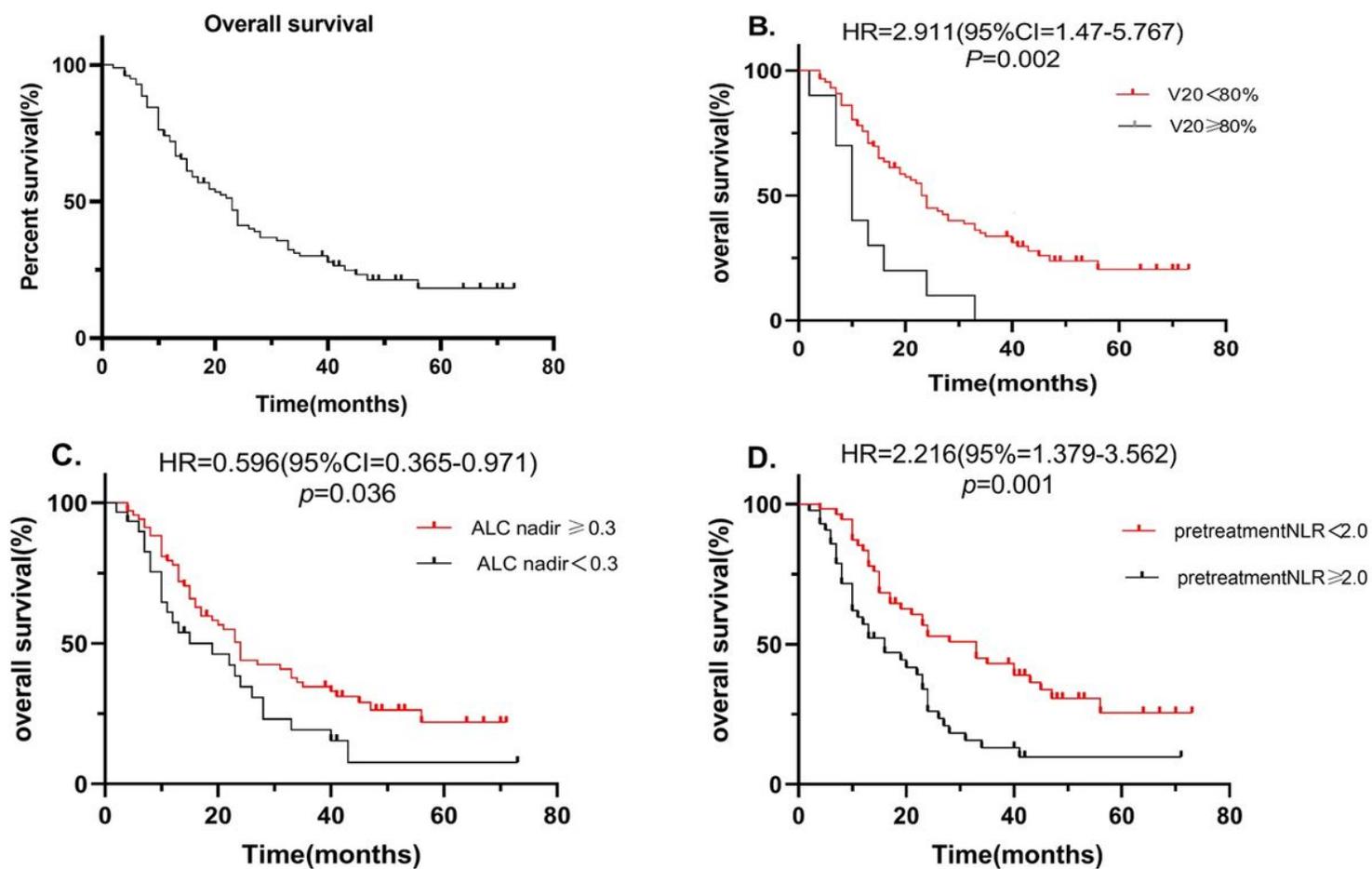


Figure 1

Kaplan-Meier curves of overall survival for the entire study cohort (A), for patients with V20 of TVB $\geq 80\%$ versus $< 80\%$ (B), for patients with ALC Nadir $\geq 0.3 \times 10^9/L$ or $< 0.3 \times 10^9/L$ (C), and for patients with Pretreatment NLR ≥ 2 or < 2 (D)