Pathological Responses of the Primary Tumor and Locoregional Lymph Nodes After Neoadjuvant Immunochemotherapy in Esophageal Squamous Cell Cancer

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Abstract

Background: The current study attempted to describe the specific patterns of pathological tumor response and locoregional node metastases from surgically resected esophageal squamous cell carcinoma after neoadjuvant immunochemotherapy (NAIC), as well as to explore the association between clinicopathological characteristics and such oncological patterns.

Methods: Fifty-one patients with cT3 or deeper esophageal squamous cell cancer underwent subtotal esophagectomy after NAIC. The NAIC regimen included intravenous administration of platinum-based and docetaxel- and taxane-based chemotherapeutics

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along with a 200 mg fixed dose of one programmed death 1 (PD-1) inhibitor, given every 3 weeks. We divided patients into tumor/nodal good-responders and poor-responders based on the pathological observation of the tumor or nodal responses. We also examined the association between clinicopathological factors and tumor/nodal responses. Further, significant baseline predictors for tumor and nodal good-responders were identified using multivariate binary logistic regression.

Results: Of the 51 patients, 68.6% achieved marked primary tumor response. Notably, 21.6% of patients achieved complete pathological response. Significant differences in treatment cycles between tumor good-responders and tumor poor-responders (P = 0.019) were observed. For locoregional nodal responses, only 33.3% of patients achieved down-staged nodal disease. Of the investigated variables, neoadjuvant cycles (odds ratio (OR): 5.271, 95% confidence interval (CI): 1.278 - 21.740, P = 0.022) and pretreatment platelets (OR: 0.979, 95% CI: 0.962 - 0.996, P = 0.017) were identified as independent predictors for good tumor and nodal responses.

Conclusions: We conclusively noted that most patients receiving NAIC were tumor good-responders, whereas only one-third of patients were nodal good-responders. Furthermore, we identified that treatment cycle number and baseline platelet counts were independent predictors of combined tumor and nodal responses.

Keywords: Esophageal squamous cell carcinoma; Neoadjuvant immunochemotherapy; Prediction model; Real-world

Introduction

Esophageal cancer (EC) has an incidence exceeding 0.6 million cases and is the sixth leading cause of death globally [1]. Despite advances in multimodal treatments, the overall prognosis for locally advanced EC is poor [2]. In Eastern Asia, esophageal squamous cell carcinoma (ESCC) is the dominant histological type of EC [3]. The rapidly fatal course of ESCC
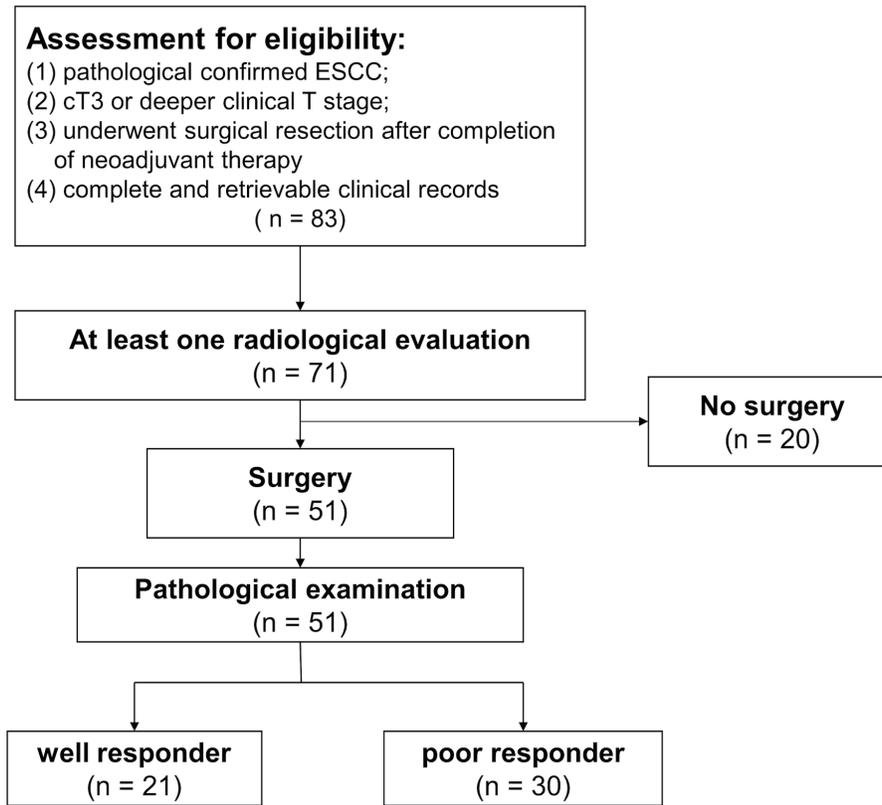
Figure 1. Flowchart of patient eligibility for inclusion. Of the 83 patients receiving neoadjuvant immunochemotherapy, 71 patients were evaluated at least once via radiological imaging. Eventually, a total of 51 patients underwent surgery. Based on the response of primary tumor and lymph nodes, patients were categorized into different groups. ESCC: esophageal squamous cell carcinoma.

results in a low 5-year overall survival [4]. Neoadjuvant chemoradiotherapy has been regarded as the standard-of-care for locally advanced ESCC [5]. Since the JCOG9907 trial, neoadjuvant chemotherapy has been regarded as a standard treatment alternative for locally advanced EC due to its superiority over adjuvant chemotherapy [6].

Clinical and histopathological responses of target tumor lesion and local-distant micrometastatic sites (e.g., regional lymph node metastasis or distant micrometastases) are considered to be independent prognosticators for EC [7-9]. The research on residual tumor patterns and locoregional lymph node metastases of the resected specimen was found to be compelling. A few studies have addressed this issue via multiple approaches [8-12]. Recent studies on patients with EC showed satisfying feasibility, efficacy, and safety of a novel treatment strategy - the neoadjuvant immunotherapy combined with chemotherapy (neoadjuvant immunochemotherapy (NAIC)) [13-18]. However, little is known about the pathological tumor response and residual nodal metastasis in patients with ESCC after NAIC.

This study aims to first describe the specific patterns of pathological tumor response and locoregional node metastases from surgically resected specimens after NAIC, and then to explore the association between clinicopathological characteristics and oncological patterns.

Materials and Methods

Patients

We conducted a retrospective study including ESCC patients who underwent NAIC followed by surgery at the Department of Thoracic Surgery, Guangdong Provincial People's Hospital from March 2019 to January 2021. The last date of follow-up in the study was October 1, 2021. The inclusion criteria were as follows: 1) pathologically confirmed ESCC; 2) cT3 or deeper clinical T stage; 3) underwent surgical resection after completion of neoadjuvant therapy; 4) complete and retrievable clinical records. Patients receiving upfront surgery or diagnosed with any M1 disease were excluded. The schematic diagram of the patient selection process is presented in Figure 1.

Preoperative workup

Before initiating NAIC, the diagnosis and clinical stage of the enrolled patients were confirmed using endoscopy-guided biopsy, contrast-enhanced computed tomography (CT), and positron emission tomography (PET). Immunotherapy based on the standard neoadjuvant chemotherapy were recommended for pa-

tients with ESCC who fit the inclusion criteria. All enrolled participants were fully informed and provided written consent. The NAIC regimen included intravenous administration of platinum-based and docetaxel-/taxane-based chemotherapeutics plus one kind of one programmed death 1 (PD-1) inhibitor, every 3 weeks intravenously. Administered PD-1 inhibitors included camrelizumab, nivolumab, pembrolizumab, sintilimab, and tislelizumab. After the completion of at least two cycles of NAIC, physical examination, routine laboratory tests, and contrast-enhanced thoracoabdominal CT or PET-CT were performed for disease evaluation. Radiological responses were recorded according to RECIST version 1.1 [19]. After a thorough evaluation of target and non-target lesions, the overall responses were denoted by complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) after a thorough evaluation of both target and non-target lesions. Patients with marked relief of cancer-related clinical symptoms and a radiological CR/PR disease were considered surgical candidates. Moreover, those with radiologically confirmed PD were deemed unfit for surgery.

Surgery

Subtotal esophagectomy with two-field lymphadenectomies were performed on medically fit patients. Our standard surgical procedure included transthoracic esophagectomy, reconstruction of the gastric tube, and cervical anastomosis between the esophageal and gastric stump.

Pathological diagnosis

The resected esophagus and lymph nodes were processed for further evaluation with a standardized protocol at our pathology department for further evaluation. Longitudinal sections of the resected specimens were embedded after formalin fixation. The specimens were then stained using hematoxylin and eosin (H&E) staining for microscopic examination and independently evaluated by two pathologists. Maximal tumor diameter, lymphovascular invasion, perineural invasion, R0 resection and lymph node positivity were assessed and reported. For study purposes, esophageal walls were divided into four layers: mucosa, submucosa, muscle and adventitia. Tumor regression grade (TRG) was evaluated per the Becker system, which is a four-tier scoring system estimating the percentage of residual tumor in relation to the macroscopically identifiable tumor bed [20]. These four grades include TRG1a: no residual tumor; TRG1b: < 10% residual tumor; TRG2: 10-50% residual tumor; TRG3: > 50% residual tumor cells with or without the signs of NAIC. According to the grade of primary tumor regression, we categorized patients into tumor good-responders (T-GRs) and tumor poor-responders (T-PRs). T-GRs were defined as patients who had TRG1a or TRG1b disease, whereas T-PR were patients who had TRG2 or TRG3 disease. Meanwhile, according to the grade of regional lymph node metastases, we categorized patients into node good-responders (N-GRs), node poor-responders (N-PRs) and node no-responders (N-NRs). N-GRs were defined as patients whose lymph node status were down-staged after neoadjuvant therapy;

N-PRs were those who had stable or progressed N stage; N-NRs were defined as patients who had no signs of positive lymph node (cN0 to pN0) from beginning to end. We further classified patients who were both T-GRs and N-GRs/N-NRs into the TN-GRs group (tumor and nodal good-responders), whereas the rest of the patients were denoted as TN-PRs (tumor and nodal poor-responders). Moreover, we also adopted pathological complete response (pCR) and tumor-associated overall survival (OS). The pCR was defined as the absence of invasive/*in situ* cancer in the primary cancer site, and OS was defined as the time from diagnosis to tumor-associated death or last follow-up.

Ethical approval

The present study was approved by the ethics committee of Guangdong Provincial People's Hospital (No. GDREC2020195H(R1)). All the procedures in this study were performed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national). This study was in compliance with the Helsinki Declaration of 1964 and later versions. Informed consent was obtained from all patients.

Statistical analysis

Descriptive data were reported as mean ± standard deviation, median (interquartile range (IQR)), or percentage. Comparisons of continuous variables were performed using Student's *t*-test and Wilcoxon-rank sum test. Categorical clinicopathological factors were compared using the Chi-square test or Fisher's exact test. $P < 0.05$ was considered statistically significant. Multivariate binary logistic regression models were built to identify independent pretreatment factors for therapeutic outcomes. These models were built via a three-step approach [21]. Forward stepwise approach was adopted. Potential confounders such as sex, height, history of smoking, history of drinking, and family oncological history were included in the models to rule out their effects. All statistical analyses were performed using the software Statistical Package for Social Science (SPSS) version 26 for Windows (SPSS, Inc, Chicago, Illinois) and R 4.0.0 (R Core Team 2020) [22]. High-quality figures were generated using the R packages.

Results

Patient characteristics

Of the 83 enrolled patients, 71 completed the planned treatment course. Eventually, 51 (71.8% in 71) were considered suitable for surgery and underwent esophagectomy. The male-to-female ratio was 4:1, and the median age of the cohort was 60 years (IQR: 54 - 65). Most primary tumors were located in the middle (45.1%) or lower portion (41.2%) of the thoracic esophagus. Overall, 45.1% of ESCC patients received ≥ 2 cycles of NAIC. The detailed clinicopathological information is presented in Table 1.

Table 1. Clinicopathological Characteristics of Esophageal Squamous Cell Cancer Patients

| Characteristics	N (%)
Sex	
Male	41 (80.4)
Female	10 (19.6)
Age (years)	
Median	60
IQR	54 - 65
BMI	
Median	22.1
IQR	20.2 - 24.6
History of smoking	
Never smoker	20 (39.2)
Previous smoker	31 (60.8)
Pack year in current or previous smokers	
Median	40
IQR	22.5 - 50
History of drinking	
Never drinker	22 (43.1)
Previous drinker	29 (56.9)
Years of drinking	
Median	30
IQR	17 - 35
Daily drinking amount (mL)	
Median	225
IQR	100 - 375
Family oncological history	
Yes	16 (31.4)
No	35 (68.6)
Tumor location	
Thoracic upper portion	6 (11.8)
Thoracic middle portion	23 (45.1)
Thoracic lower portion	21 (41.2)
Esophagogastric junction	1 (2.0)
Clinical T stage	
T3	31 (60.8)
T4a	20 (39.2)
Clinical N stage	
N0	20 (39.2)
N1	29 (56.9)
N2	2 (3.9)
Clinical TNM stage	
II	13 (25.5)
III	18 (35.3)
IVA	20 (39.2)

Table 1. Clinicopathological Characteristics of Esophageal Squamous Cell Cancer Patients - *(continued)*

Characteristics	N (%)
Preoperative cycles	
Two	28 (54.9)
Three	21 (41.2)
Four	2 (3.9)
pCR status	
pCR	11 (21.6)
non-pCR	40 (78.4)
Neoadjuvant pathological tumor depth	
T0	11 (21.6)
Tis	9 (17.6)
T1a	5 (9.8)
T1b	2 (3.9)
T2	8 (15.7)
T3	15 (29.4)
T4a	1 (2.0)
Neoadjuvant pathological lymph node	
N0	34 (66.7)
N1	12 (23.5)
N2	3 (5.9)
N3	2 (3.9)
Neoadjuvant pathological stage	
I	27 (52.9)
II	7 (13.7)
IIIA	6 (11.8)
IIIB	9 (17.6)
IVA	2 (3.9)
Lymphovascular invasion	
Yes	4 (7.8)
No	47 (92.2)
Perineural invasion	
Yes	4 (7.8)
No	47 (92.2)
R0	
Yes	5 (9.8)
No	46 (90.2)

BMI: body mass index; IQR: interquartile range; pCR: pathological complete response.

Pathological response of primary tumors and association with clinicopathological factors

The pathological tumor response pattern is presented in Figure 2a. Tumor invasion depths among the neoadjuvant recipients were at least T3 before initiation of treatment. Of the selected
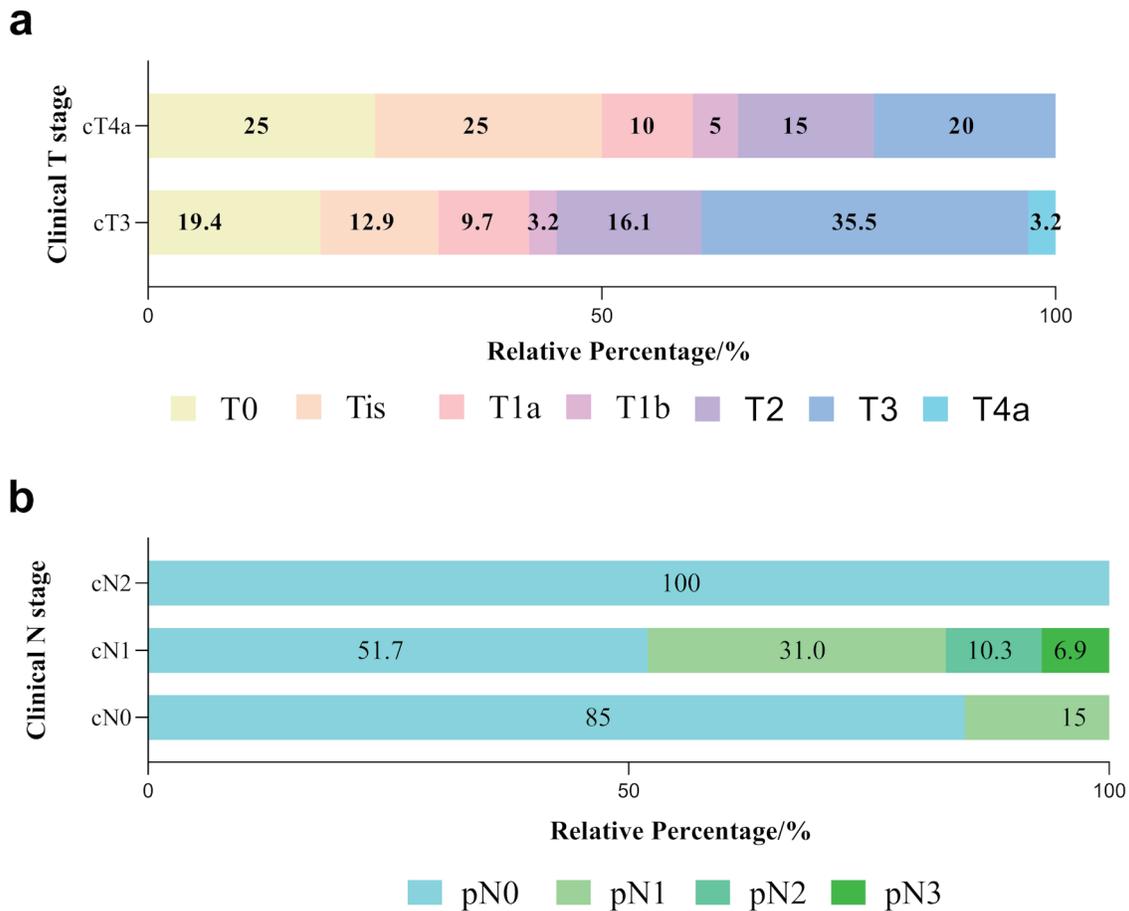
Figure 2. Tumor and nodal responses in ESCC patients with various clinical stages. (a) Differences in pathological T stage between cT3 and cT4a patients. Various color blocks represent depths of tumor invasion. (b) Differences in pathological lymph node response (represented by different color blocks) among cN0, cN1 and cN2 groups after neoadjuvant immunochemotherapy. ESCC: esophageal squamous cell carcinoma.

surgical candidates, tumor invasion depths were limited to the muscular layer in 68.6% of patients. The frequently involved residual tumor sites were identified in the mucosa and submucosa. Among the cT3 group, 45.2% of patients (n = 14) achieved TRG1a/1b; while 65% (n = 20) of patients in cT4 group reached TRG1a/1b. Notably, 11 patients (21.6%) had complete pathological response disease (pCR), while nine patients had carcinoma *in situ* disease. Less than 10% residual tumor was observed in 52.9% of the resected specimens (n = 27).

We compared the clinicopathological factors between T-GRs and T-PRs. Significant differences were observed in age (P = 0.049), pretreatment neutrophil-to-lymphocyte (P = 0.006), pretreatment neutrophil ratio (P = 0.003), pretreatment lymphocyte ratio (P = 0.009), pretreatment neutrophil count (P = 0.036), neoadjuvant cycles (P = 0.017), pCR (P = 0.001), post-treatment primary tumor SUVmax (P = 0.042), lymphovascular invasion (P = 0.016), perineural invasion (P = 0.016), and ypTNM (P < 0.001). Notably, the proportion of T-GRs was significant higher in the group that received three cycles of NAIC than those with only two cycles (76.2% in three cycles vs. 35.7% in two cycles, P = 0.017 (< 0.05)). All of the T-GRs

had both negative lymphovascular and perineural invasion, compared to 18.2% positive findings (n = 4) in T-PRs (P = 0.016). No significant association between immunohistochemical markers and T-GRs/PRs were observed.

Neoadjuvant locoregional lymph node metastases and their association with other clinicopathological factors

The pattern of locoregional lymph nodes metastases after NAIC was depicted (Fig. 2b). Patients with clinical N stage of N0-2 were enrolled. Of the 20 cN0 patients at pretreatment phase, three (15%) had pN1 disease after surgery. Notably, 15 out of 29 (51.7%) cN1 patients achieve pN0 status. However, five patients (17.2%) experienced more lymph node metastases. In contrast, two patients with cN2 before NAIC were free from node metastases. Only 29.4% of patients achieved downstaged N disease.

We compared the clinicopathological factors between N-GRs and N-PRs. Significant differences were observed in the pretreatment neutrophil-to-lymphocyte ratio (P = 0.006),

Table 2. Univariate and Multivariate Binary Logistic Regression of Pretreatment Clinicopathological Factors for Prediction of Tumor and Nodal Responses

| Variable | Poor-responder vs. good-responder | | | |
| | Univariate | | Multivariate | |
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Sex	1.562 (0.389 - 6.269)	0.529		
Age (years)	4.0 (1.225 - 13.056)	0.022	1.086 (0.984 - 1.198)	0.102
BMI	1.185 (0.981 - 1.431)	0.078	1.291 (0.994 - 1.678)	0.056
Pretreatment clinical T stage	0.923 (0.294 - 2.898)	0.891		
Pretreatment clinical N stage	1.115 (0.407 - 3.052)	0.833		
Pretreatment clinical stage	1.335 (0.663 - 2.688)	0.418		
Cycles	4.875 (1.428 - 16.641)	0.011	5.271 (1.278 - 21.740)	0.022
Pretreatment neutrophil-to-lymphocyte ratio	1.433 (0.929 - 2.210)	0.039	1.587 (0.860 - 2.930)	0.14
Pretreatment while blood cells ($\times 10^9$/L)	0.979 (0.745 - 1.288)	0.88		
Pretreatment hemoglobin (g/L)	0.990 (0.957 - 1.024)	0.557		
Pretreatment red blood cells ($\times 10^{12}$/L)	0.477 (0.142 - 1.602)	0.231		
Pretreatment platelets ($\times 10^9$/L)	0.988 (0.976 - 1.000)	0.053	0.979 (0.962 - 0.996)	0.017
Pretreatment neutrophils ($\times 10^9$/L)	1.095 (0.791 - 1.515)	0.586		
Pretreatment lymphocytes ($\times 10^9$/L)	0.462 (0.166 - 1.290)	0.141		
Pretreatment monocytes ($\times 10^9$/L)	0.346 (0.027 - 4.378)	0.412		

CI: confidence interval; BMI: body mass index.

pretreatment neutrophil ratio (P = 0.003), pretreatment lymphocyte ratio (P = 0.012), pretreatment neutrophil count (P = 0.036), pretreatment erythrocyte count (P = 0.025), posttreatment primary lesion SUVmax (P = 0.001), lymphovascular invasion (P = 0.026), perineural invasion (p = 0.029), pCR (P = 0.034), R0 (P = 0.009), and ypTNM (P < 0.001). Notably, the proportion of N-PRs was significantly higher in those with negative lymphovascular invasion (74.4% N-PRs vs. 25.6% N-GRs). Moreover, a higher proportion of R0 rate was higher in N-GRs (61.5%) compared to that in N-PRs (38.5%) (P = 0.009).

Combined primary tumor and residual lymph node response

To further explore the pretreatment clinicopathological predictors of T-responders and N-responders, we performed binary logistic regression analyses. Univariate analyses revealed that age (odds ratio (OR): 4.0, 95% confidence interval (CI): 1.225 - 13.056, P = 0.022), neoadjuvant treatment cycles (OR: 4.875, 95% CI: 1.428 - 16.641, P = 0.011), pretreatment neutrophil-to-lymphocyte ratio (OR: 1.433, 95% CI: 0.929 - 2.210, P = 0.039) were significantly associated with combined tumor and nodal responses (Table 2). After adjusted for potential cofounders, further multivariate regression analyses identified neoadjuvant cycles (OR: 5.271, 95% CI: 1.278 - 21.740, P = 0.022) and pretreatment platelets (OR: 0.979, 95% CI: 0.962 - 0.996, P = 0.017) as independent predictors for good tumor and nodal responses (Fig. 3).

Prognostic significance of combined pathological responsiveness

We conducted a survival analysis to investigate differences in tumor-associated OS between TN-GRs and TN-PR groups. Significantly higher OS was observed in TN-GRs than that in TN-PRs (P < 0.05, Fig. 4).

Subgroup survival analysis

Additionally, we conducted survival analyses based on patients' pretreatment clinical TNM stages. No significant differences in clinical T stage (Supplementary Material 1, www.wjon.org), clinical N stage (Supplementary Material 2, www.wjon.org), and clinical TNM stage (Supplementary Material 3, www.wjon.org) were found.

Discussion

Currently, although immunotherapy combined with chemotherapy has not been considered as standard-of-care for ESCC, several studies have proved the satisfying efficacy and safety of NAIC [15-18]. In this pilot study, we described the pathological response of primary tumor and locoregional lymph node metastases of locally advanced ESCC patients receiving NAIC. Based on the differences in response, we classified patients into T-/N-GRs or -PRs. Further, we also identified independent preoperative clinicopathological factors which pre-

Variables	p value	Odds ratio	
Age	0.102	1.086(0.984−1.198)	
BMI	0.056	1.291(0.994−1.678)	
Cycles	0.022	5.271(1.278−21.740)	
Pre-N:L	0.140	1.587(0.860−2.930)	
Pre-Platelets	0.017	0.979(0.962−0.996)	

Odds ratio

0   5   10   15   20

Favor poor responders   Favor well responders
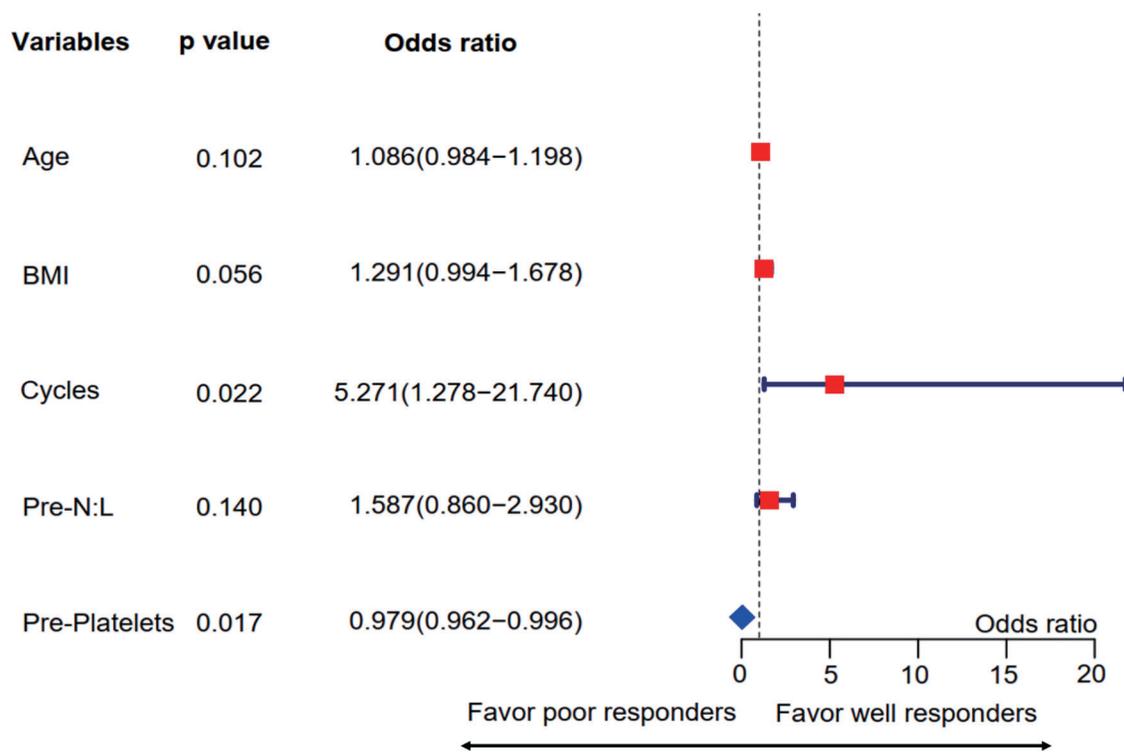
Figure 3. Multivariate regression analysis for pretreatment predictors associated with combined tumor and nodal responses. Variables with P values ≤ 0.1 in the univariate analysis were included in this step. The forest plot showed that treatment cycles and pretreatment platelet counts were independent predictors for combined TN responses. Pre-N:L: pretreatment neutrophil to lymphocyte ratio; Pre-platelets: pretreatment platelet count; BMI: body mass index.

p<0.05

Pathological responsiveness
- Poor responder
- Well responder
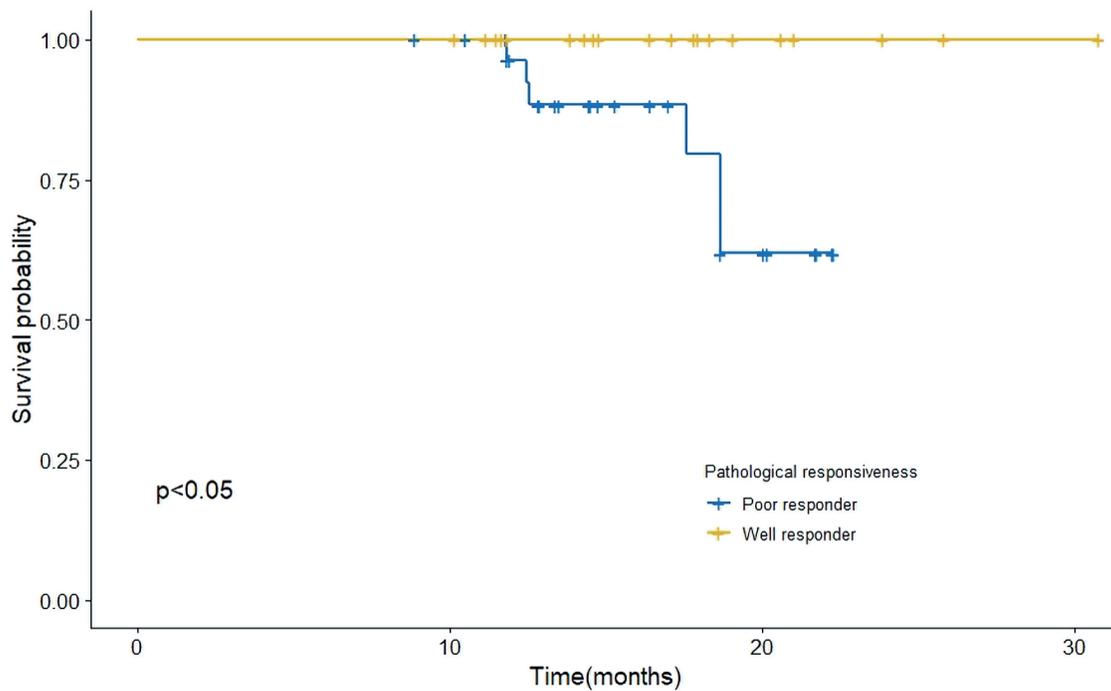
Figure 4. Kaplan-Meier curves for overall survival in the surgical cohort. The difference in OS between pathologically good-responders (TN-GRs) and poor-responders (TN-PRs). Significant difference in overall survival between these groups was observed. OS: overall survival.

dicted the overall responses of the primary tumor and regional lymph nodes. To our knowledge, this study was the first to evaluate the patterns of pathological tumor and nodal response after NAIC, and their association with clinicopathological characteristics.

It has been shown that residual viable tumor cells in both primary sites and regional lymph nodes after neoadjuvant therapy were observed to be significantly associated with the prognosis of ESCC [23]. The present study showed significant regressions in the primary tumor after NAIC in most patients with ESCC. The most frequently involved sites were mucosa and submucosa. These findings were consistent with those discovered observed in patients with ESCC receiving neoadjuvant chemoradiotherapy [10, 11]. Notably, 11 patients (21.6%) achieved tumor pathological complete response (T-pCR), which was lower than those reported by recent phase IB-II studies [15-18]. A plausible explanation for this difference is that compared to other clinical trials with idealized designs, this real-world study adopted relaxed inclusion criteria which reflected a more realistic treatment environment.

Subsequently, we classified patients into different groups based on the pathological response of primary tumors and lymph nodes. In terms of T-responders, 76.2% of patients who received three-cycle NAIC achieved a good response, which was twice higher as those who received two-cycle NAIC (P = 0.017). Furthermore, the multivariate binary logistic regression indicated that the neoadjuvant cycle number was an independent predictor for primary tumor response. Those who received three cycles of NAIC were three times more likely to become T-GRs, compared to those that receiving two cycles. A possible explanation for this enhanced efficacy is that an increase in the cycle numbers could lead to an elevated concentration of immunochemotherapy. Moreover, the longer duration of response in immunotherapy-treated groups may be another possible explanation [24, 25]. The three-cycle NAIC could potentially achieve more persistent responses due to a longer neoadjuvant period. Previous studies have reported favorable survival outcomes in tumor patients receiving more cycles of neoadjuvant or adjuvant therapy [26-28]. However, no studies have investigated the differences in the primary tumor response in ESCC patients with varying numbers of neoadjuvant cycles. This study was the first to reveal a relationship between neoadjuvant cycle number and primary tumor response, which could provide a reference for designing trial regimens in the future.

The lymph node response to neoadjuvant therapy was crucial in subsequently followed-up patients with ESCC, especially those with tumor recurrence and metastases [9, 29]. A recent study reported that the predictive values of lymph node response to neoadjuvant therapy were more reliable than those of tumor response [29]. Interestingly, the pattern of lymph node metastases after NAIC was inconsistent with that of the primary tumor. Although lymph node downstaging was observed in 17 (33%) patients, there remain a significant proportion of the patients who had no lymph node response (33%) or even up-staging of the disease (33%). Hamai et al reported that 49.3% of patients had down-staged nodal disease after neoadjuvant chemoradiotherapy. It suggested that the synergistic effect of immunotherapy on chemotherapy may be weaker than

on radiotherapy; similar findings were reported by Hsu et al [9]. The distinct response to NAIC between primary tumor and lymph nodes could be attributed to their structural and micro-environmental heterogeneity, such as types and abundance of immune cells, oxygen levels, and cytokines [30-32].

Further, multivariate analyses of the combined primary tumor and lymph nodes responses also indicated that the neoadjuvant cycle number and baseline platelet counts were independent predictors. Patients who received a three-cycle regimen were five times more likely to be TN-GRs compared to those who received a two-cycle regimen. Wu et al reported that no significant association between pretreatment platelet and tumor/nodal responses was observed [33]. Other researchers found that elevation of pretreatment platelets was significantly correlated with better neoadjuvant response, but not OS [34]. However, McLaren et al reported that elevated hematological parameters that incorporate pretreatment platelet count were predictive of worse OS [35]. The discrepancies among these studies may be explained by the high numerical difference in baseline platelets among individuals, which could lead to unstable data. Moreover, the sample sizes in these studies, including the current study (51 - 306), were relatively too small to decisively conclude on the predictive value of the baseline platelet counts. Studies with larger cohorts are needed in the future to confirm this paradoxical observation.

There were some limitations in our study. First, this retrospective study was conducted at a single institution which hampered the clinical promotion and popularization of the findings. Secondly, as discussed earlier, the sample size was too small and large-scale, prospective, multi-centered studies were needed in the future.

In conclusion, we found good response occurred in primary tumors among most ESCC patients receiving NAIC. Good nodal response, on the contrary, only occurred in a small proportion of patients. Furthermore, we identified neoadjuvant cycle number and baseline platelet counts were independent predictors of combined tumor and nodal responses.

Supplementary Material

Suppl 1. Kaplan-Meier curves for overall survival in patients with clinical T3 stage and clinical T4a stage.

Suppl 2. Kaplan-Meier curves for overall survival in patients with clinical N0 stage and clinical N+ stage.

Suppl 3. Kaplan-Meier curves for overall survival in patients various clinical TNM stages.

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Financial Disclosure

Informed Consent

Informed consent was obtained from all patients.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Shu Jie Huang, Dan Tian and Si Chao Wang: conceptualization, data curation, formal analysis, investigation, methodology, roles/writing - original draft, writing - review and editing. Rui Jie Zeng, Yue Jiao Dong, Liang Li Hong, Han Sheng Wu, Fang Ping Xu, Dong Kun Zhang, Liang Xie, Hai Yu Zhou, Ji Ming Tang, Xiao Song Ben, Gang Chen, Ri Xin Chen: software, validation, visualization, investigation, methodology, roles/writing - original draft; writing - review and editing. Yong Tang and Gui Bin Qiao: funding acquisition, project administration, resources, supervision, roles/writing - original draft, writing - review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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