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Neutrophil–to–lymphocyte Ratio and Use of Antibiotics Associated With Prognosis in Esophageal Squamous Cell Carcinoma Patients Receiving Immune Checkpoint Inhibitors

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Abstract. *Background/Aim: This study explored the prognostic significance of the neutrophil–to–lymphocyte ratio (NLR) and use of antibiotics in advanced esophageal squamous cell carcinoma (ESCC) patients receiving immune checkpoint inhibitors (ICIs). Patients and Methods: Patients were enrolled from two referral centers in Taiwan. Clinical benefit was defined as complete response, partial response, or a stable disease for ≥6 months via Response Evaluation Criteria In Solid Tumors 1.1. Clinicopathological factors' impact on overall survival (OS) and progression-free survival (PFS) was analyzed via Cox proportional hazards model. Results: Forty-nine patients were enrolled. The median PFS and OS were 1.8 and 6.1 months, respectively. The median NLR at baseline was 6.40, and 21 patients received antibiotics. Both high NLR and use of antibiotics were associated with inferior PFS (p=0.028 and p<0.001, respectively) and OS (p<0.001 and p<0.001, respectively) in multivariate analysis. Conclusion: High NLR and use of antibiotics were associated with inferior survival in advanced ESCC patients receiving ICIs.*

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Esophageal cancer (EC) is the sixth most lethal malignancy in the world, accounting for 400,000 deaths in 2012 (1). Esophageal squamous cell carcinoma (ESCC) is the predominant histology subtype in eastern and central Asia, where esophageal cancer is relatively endemic (2). Despite the advancements that have been made in surgery, chemotherapy, and radiotherapy (3, 4), the prognosis for patients diagnosed with esophageal cancer remains unsatisfactory. Patients with recurrent or metastatic ESCC, for which the indicated therapy is systemic chemotherapy, have a median survival time in the range of 8 to 11 months (3-5).

Blockade of immune checkpoints, especially programmed cell death protein-1 (PD-1) and PD ligand 1 (PD-L1), has become a new paradigm in cancer therapy and have demonstrated significant clinical benefit in an increasing number of cancer types. In KEYNOTE-181, pembrolizumab outperformed 2nd-line chemotherapy with a significant improvement in overall survival (OS) of EC patients with high PD-L1 expression (*i.e.*, combined positive score ≥10) and a clinically meaningful improvement in OS of ESCC patients (6). Another phase III trial, ATTRACTION-3, also demonstrated that nivolumab, compared to 2nd-line chemotherapy, significanty improved OS in patients with advanced ESCC (7). Overall, anti-PD-1 therapy may soon become a standard-of-care for recurrent or metastatic EC.

Inflammatory response in the tumor microenvironment may promote tumor progression (8). The neutrophil–to–lymphocyte ratio (NLR), calculated from the numbers of peripheral white blood cells, is a simple index of systemic inflammatory response. The NLR has been proposed as a prognostic factor for patients with cancer. In multiple cancer types evaluated at different disease stages or under various treatment modalities, a high NLR has been demonstrated to be associated with an inferior

prognosis (9). Recently, the prognostic significance of the NLR was investigated in patients receiving cancer immunotherapy. In patients with non-small-cell lung cancer (NSCLC), melanoma, and renal cell carcinoma (RCC) who were treated with immune checkpoint blockade-based therapy, a high NLR was associated with a poor prognosis (10-12). The NLR has been previously studied in patients with ESCC being treated with conventional therapy, but the prognostic significance of the NLR in patients with ESCC treated with PD-1/PD-L1-blockade-based immunotherapy has never been reported.

The gut microbiota has been shown to play a role in shaping the systemic immune responses of its host (13). In mouse models, specific members of the gut microbiota have been demonstrated to influence the efficacy of immune checkpoint inhibitors (14, 15). This observation was recently verified in patients with NSCLC and RCC who were treated with PD-1/PD-L1-blockade-based immunotherapy; the types of gut bacteria at baseline were consistently different between patients who were responsive and those who were nonresponsive to the therapy (16). Moreover, the use of antibiotics, which can change the composition of the gut microbiome, was associated with poor prognoses in patients with NSCLC, RCC, and urothelial carcinoma who were treated with PD-1/PD-L1-blockade-based immunotherapy (16). Whether this phenomenon can be extrapolated to ESCC patients is currently unclear.

To investigate the prognostic and predictive significance of the NLR and the use of antibiotics in patients with ESCC receiving PD-1/PD-L1-blockade-based immunotherapy, we performed this retrospective analysis, enrolling patients from two medical centers in Taiwan.

Patients and Methods

Study cohort. Patients with recurrent or metastatic EC who were treated with PD-1/PD-L1-blockade-based therapy between August 1, 2015, and December 31, 2017, were retrospectively identified from the National Taiwan University Hospital, Taipei, Taiwan, and China Medical University Hospital, Taichung, Taiwan. Patients diagnosed as having squamous cell carcinoma of the esophagus were enrolled; whereas patients with adenocarcinoma, small cell carcinoma, or neuroendocrine carcinoma were ineligible. PD-1/PD-L1-blockade-based immunotherapy was administered every 2 to 4 weeks until the disease progressed or intolerable toxicities occurred. Follow-up imaging studies were conducted every 8 to 12 weeks to evaluate tumor response. Data concerning pertinent clinicopathological characteristics, blood test results, survival status, and additional cancer therapies were retrieved retrospectively from medical records. The study was approved by our institutional Research Ethical Committee.

Definition of the NLR and the use of antibiotics. The baseline NLR was calculated by dividing the neutrophil count by the lymphocyte count, using the data obtained within 7 days prior to the start of treatment. Use of antibiotics was defined as receiving systemic antibiotics for any indication between 2 months prior to and 1 month after starting immunotherapy.

Statistics. Follow-up data were compiled until February 28, 2018: the cutoff date. The primary endpoint was whether the NLR or the use of antibiotics predicted the prognosis of patients with recurrent or metastatic ESCC receiving PD-1/PD-L1-blockade-based therapy. Descriptive statistics were used for analyzing the baseline clinicopathological characteristics. Tumor response was evaluated according to the Response Evaluation Criteria In Solid Tumours 1.1 (17). Clinical benefit was defined as complete response, partial response, or a stable disease for more than 6 months. Progression-free survival (PFS) was defined as the time between the date of starting PD-1/PD-L1-blockade-based therapy and the date of progressive disease or death or final follow-up (censored). OS was defined as the time between the date of starting PD-1/PD-L1-blockade-based therapy and the date of death or final follow-up (censored). The Kaplan–Meier (KM) method was used to estimate survival curves, and the log-rank test was used to compare between survival curves. Clinicopathological factors, the dichotomized baseline NLR (high *versus* low with respect to the median), and the use of antibiotics were analyzed for their impacts on the patients' OS and PFS using the univariate Cox proportional hazards model. The statistically significant variables ($p \leq 0.05$) were then analyzed using the multivariate Cox proportional hazards model. All data analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and the survival curves were plotted by GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA).

Results

Patient characteristics. Forty-nine patients were identified. Most patients were male (98%) and had a favorable Eastern Cooperative Oncology Group (ECOG) performance status (0 or 1, 92%). Thirty-three patients had recurrent disease, and 16 patients had de novo metastatic disease. Twenty-five patients (51%) had a tumor burden of ≥2 organ sites, and 35 (71%) had visceral organ metastasis. Twenty-eight patients (57%) had received one prior systemic therapy, whereas 19 patients (39%) had received ≥2 lines of prior systemic therapy for recurrent or metastatic disease. The response rate for evaluable patients was 10.2% [95% confidence interval (CI)=1.6%-18.8%]. The clinicopathological characteristics of the enrolled patients are summarized in Table I.

NLR and the use of antibiotics. Blood test results are summarized in Table II. The median white blood cell count was 7,170 per μl (interquartile range=5,015-9,825 per μl). The median NLR was 6.40 (interquartile range=3.06-10.48). We divided the patient population into groups having high and low NLRs according to the median NLR level, and we found that more high-NLR patients were treated with PD-1 or PD-L1 blockade alone than low-NLR patients ($p=0.030$). On analysis of the association of response rate or clinical benefit, we found that low-NLR patients were more likely to be clinically benefitted than high-NLR patients ($p=0.018$).

Table I. *Baseline demographics and clinical characteristics (N=49).*

Characteristic	No. (%)
Median age, years (range)	56.7 (37.2-82.8)
ECOG performance status	
0-1/>1	45 (92)/4 (8)
Gender	
Male/Female	48 (98)/1 (2)
Primary esophageal cancer	
Cervical and upper thoracic	16 (33)
Middle thoracic	16 (33)
Lower thoracic	17 (35)
Differentiation	
Well/Moderate	2 (4)/23 (47)
Poor/Unknown	12 (24)/12 (24)
Disease status	
Recurrent/*De novo* metastatic	33 (67)/16 (33)
Esophagectomy	
Yes/No	19 (39)/30 (61)
Tumor burden - involved sites	
1/>1	24 (49)/25 (51)
Treatment	
PD-1/PD-L1 blockade alone	30 (61)
PD-1/PD-L1-blockade-based combination	19 (39)
Prior lines of therapy for recurrent/metastatic disease	
0/1	2 (4)/28 (57)
2/≥3	15 (31)/4 (8)
Best response	
Complete response/Partial response	0 (0)/5 (10)
Stable disease/Progressive disease	15 (31)/26 (53)
Not evaluable	3 (6)
Clinical benefit response[a]	
Yes	11 (22)
No	38 (78)

[a]Clinical benefit rate: complete response, partial response and stable disease ≥6 months. ECOG: Eastern Cooperative Oncology Group; PD-1/PD-L1: programmed cell death protein-1/PD ligand 1.

Table II. *Baseline blood characteristics.*

Characteristics (median, interquartile)	Total (N=49)
White blood cell counts (per μl)	7170 (5015-9825)
Neutrophils (per μl)	5882 (3488-7562)
Lymphocytes (per μl)	889 (673-1120)
Neutrophil-to-lymphocyte ratio	6.40 (3.06-10.48)
Monocyte (per μl)	588 (390-771)
Eosinophil (per μl)	91 (52-201)
Platelet (K per μl)	260 (195-354)
Albumin (g/dl)	3.9 (3.5-4.3)
Lactate dehydrogenase (U/l)	172 (145-229)

Twenty-one patients (43%) were identified as receiving systemic antibiotics between 2 months before and 1 month after starting anti-PD-1/PD-L1-blockade-based therapy. Patients who had received antibiotics, compared with those who had not, had poorer ECOG performance status (p=0.028) and a lower chance of response and clinical benefit (p=0.023 and 0.014, respectively). The median duration of systemic antibiotics use was 10 days (range=1-43 days). Fourteen patients (67%) were prescribed β-lactam ± β-lactamase inhibitor. The most common indication for the use of antibiotics was pneumonia (46%), followed by prophylaxis for surgery or an invasive procedure (25%).

Survival analysis. At the data cutoff date (February 28, 2018), the median follow-up was 16.4 months (range=2.2-29.9 months). Median PFS and OS were 1.8 (95%CI=1.0-2.6) and 6.1 (95%CI=4.2-8.0) months, respectively. The median PFS for patients with low and high NLRs (<median *vs*. ≥median) was 2.8 months (95%CI=0.3-5.3) and 1.4 months (95%CI=1.3-1.5), respectively (p=0.001). The median OS for patients with low and high NLRs was 10.4 months (95%CI=9.8-11.0) and 3.0 months (95% CI=2.4-3.6), respectively (p<0.001). The PFS and OS curves for all patients and the patients with low and high NLRs are shown in Figure 1A-D.

The median PFS for patients without and with the use of antibiotics was 2.8 months (95%CI=1.1-4.5) and 1.3 months (95%CI=1.1-1.5), respectively (p<0.001). The median OS for patients without and with the use of antibiotics was 10.4 months (95%CI=8.0-12.8) and 3.0 months (95%CI=1.5-4.5), respectively (p<0.001). The PFS and OS curves for patients without and with the use of antibiotics are shown in Figure 1E and F.

We conducted an exploratory analysis, incorporating a high NLR and the use of antibiotics as risk factors for survival outcomes. The median PFS for patients having 0, 1, or 2 risk factors was 5.8 months (95%CI=2.1-9.5), 2.0 months (95%CI=0.5-3.5), and 1.1 months (95%CI=0.7-1.5), respectively (p<0.001); the median OS for patients having 0, 1, or 2 risk factors was 11.7 months (95%CI, 4.8-18.6), 6.1 months (95%CI=4.6-7.6), and 1.5 months (95%CI=0.9-2.1), respectively (p<0.001). The PFS and OS curves for patients classified according to the risk factors are shown in Figure 1G and H.

Prognostic significance of the NLR and the use of antibiotics. In univariate analysis, poor ECOG performance status (p=0.001), large tumor burden (p=0.030), PD-1/PD-L1 blockade alone (p=0.027), high NLR (p=0.002), and the use of antibiotics (p<0.001) were statistically associated with inferior PFS; poor ECOG performance status (p<0.001), large tumor burden (p=0.001), high NLR (p<0.001), and the use of antibiotics (p<0.001) were statistically associated with inferior OS.

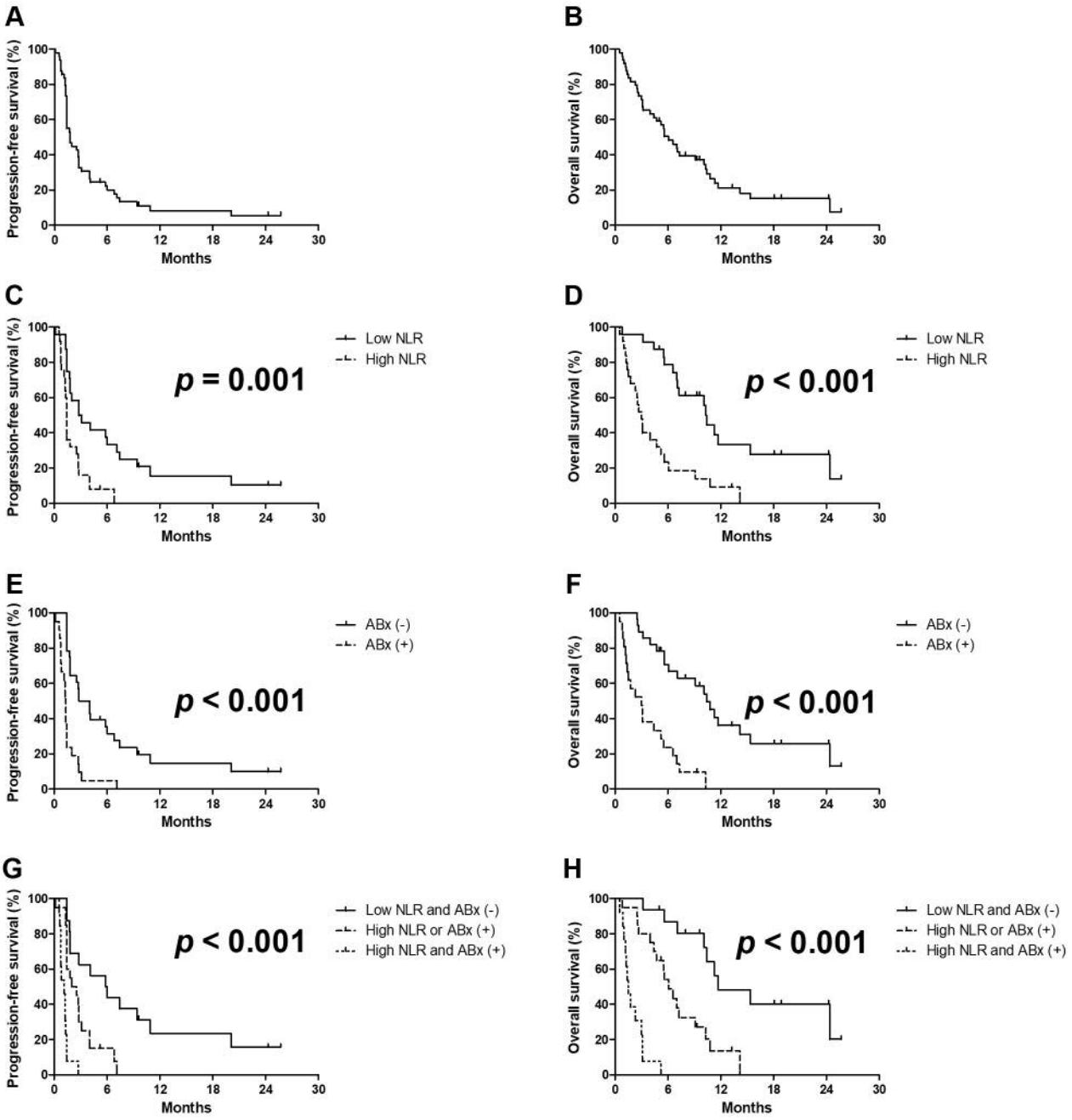

Figure 1. *Kaplan–Meier curves of progression-free survival (A, C, E) and overall survival (B, D, F) for the entire study cohort (A, B), for patients with high versus low neutrophil-to-lymphocyte ratio (NLR, dichotomized by median) (C, D), for patients with or without the use of antibiotics (ABx) (E, F), and for patients according to the risk factors (NLR and ABx) (G, H).*

Multivariate analysis included all the significant factors identified in the univariate analysis, and the results were that PD-1/PD-L1 blockade alone (p=0.044), high NLR (p=0.028), and the use of antibiotics (p<0.001) were associated with inferior PFS; large tumor burden (p=0.004), high NLR (p<0.001), and the use of antibiotics (p<0.001) were statistically associated with inferior OS. The univariate and multivariate analyses performed using the Cox proportional hazards model are summarized in Table III.

Table III. *Univariate and multivariate analyses of progression-free survival and overall survival (Cox proportional hazards model).*

	Univariate				Multivariate			
	Progression-free survival		Overall survival		Progression-free survival		Overall survival	
Variable	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
ECOG PS								
0-1 (ref.) *vs.* >1	6.25 (2.08, 18.79)	0.001	15.88 (4.15, 60.79)	<0.001	1.22 (0.37, 3.98)	0.741	3.31 (0.83, 13.20)	0.091
Primary[a]								
C and U	1.00	0.982	1.00	0.680				
M	1.02 (0.49, 2.13)	0.961	1.23 (0.56, 2.71)	0.602				
L	1.07 (0.53, 2.17)	0.852	1.42 (0.54, 3.12)	0.382				
Grade								
Well and unknown	1.00	0.870	1.00	0.917				
Moderate	1.20 (0.59, 2.43)	0.613	1.18 (0.54, 2.57)	0.677				
Poor	1.19 (0.52, 2.69)	0.686	1.11 (0.47, 2.62)	0.820				
Disease status								
Recurrent	1.00	0.519	1.00	0.352				
De novo metastatic	1.23 (0.65, 2.32)		1.38 (0.70, 2.70)					
Esophagectomy								
No (ref.) *vs.* Yes	1.00 (0.53, 1.90)	0.996	0.93 (0.47, 1.83)	0.831				
Tumor burden								
Site=1 (ref.) *vs.* >1	1.98 (1.07, 3.68)	0.030	3.30 (1.66, 6.56)	0.001	1.34 (0.67, 2.68)	0.416	3.30 (1.48, 7.37)	0.004
Treatment								
Combo (ref.) vs.	1.00	0.027	1.00	0.437	1.00	0.044	1.00	0.263
PD-1/PD-L1 alone	2.09 (1.09, 4.03)		1.30 (0.67, 2.53)		2.19 (1.02, 4.71)		0.61 (0.25, 1.46)	
Prior therapies								
<2 (ref.) *vs.* ≥2 lines	1.25 (0.69, 2.28)	0.468	1.51 (0.78, 2.90)	0.221				
NLR (median)								
<6.4 (ref.) *vs.* ≥6.4	2.80 (1.45, 5.40)	0.002	3.72 (1.89, 7.38)	<0.001	2.28 (1.09, 4.74)	0.028	6.31 (2.38, 16.77)	<0.001
Antibiotic use								
No (ref.) *vs.* Yes	3.91 (2.07, 7.42)	<0.001	5.06 (2.41, 10.63)	<0.001	5.11 (2.42, 10.82)	<0.001	5.88 (2.55, 13.55)	<0.001

[a]C: Cervical; U: upper thoracic; M: middle thoracic; L: lower thoracic; ECOG PS: Eastern Cooperative Oncology Group performance status.

Discussion

Immunotherapy, especially PD-1/PD-L1 blockade, has changed the landscape of cancer therapy for many cancer types, including ESCC (6, 7). In this study, we retrospectively analyzed a cohort of recurrent or metastatic ESCC patients who were treated with PD-1/PD-L1-blockade-based therapy and found that both a high NLR and the use of antibiotics were statistically significant prognostic factors associated with inferior PFS and OS.

The use of the NLR as a prognostic factor for patients with cancer has been extensively investigated. Although the optimal cutoff ratio for the NLR remains unknown, a high NLR has been repeatedly demonstrated to correlate with a poor prognosis in multiple cancer types when patients are treated with either locoregional therapies or systemic chemotherapy (9). Our findings, consistent with recent publications concerning NSCLC, melanoma, and RCC patients who were treated with immune checkpoint blockade-based therapy, support the premise that a high baseline NLR is associated with poor treatment outcomes in patients with cancer who receive immunotherapy (10-12). In one study of NSCLC patients who were treated with PD-1 blockade, the NLR at 6 weeks after treatment initiation was even discovered to be a prognostic marker, implying that the posttreatment NLR may also have prognostic significance in some cancer types (18).

The use of antibiotics to treat infections has improved the outcome of patients with leukemia (19). However, in patients with chronic lymphocytic leukemia and relapsed lymphoma who received a cyclophosphamide-containing first-line therapy and a cisplatin-containing regimen, respectively, those who were exposed to anti-gram-positive antibiotics had lower overall response, inferior PFS, and even reduced OS (20). It has been hypothesized that the use of broad-spectrum antibiotics leads to the proliferation of pathogenic bacteria, translocation of bacteria across disrupted intestinal mucosa, and subsequent infection (21). Recently, the gut microbiota

has been discovered to modulate the efficacy of PD-1/PD-L1-blockade-based therapy in patients with NSCLC and RCC (16). The use of systemic antibiotics, which cause dysbiosis of gut microbiota, has been recognized to be a poor prognostic factor for patients with NSCLC and RCC who are receiving PD-1/PD-L1-blockade-based therapy (16, 22). However, the use of antibiotics was not associated with the efficacy of nivolumab or prognosis in a smaller NSCLC cohort (23).

In ordinary clinical practice, the use of antibiotics is commonly seen in patients who are in a fragile condition and have comorbidities that contribute to a high risk of infections. This type of patients tends to have leukocytosis and a high NLR. However, in our multivariate analysis, the use of antibiotics and a high NLR were independent prognostic factors for both PFS and OS. This observation implies that although the use of antibiotics and a high NLR may be associated with the same group of patients with "unfavorable prognosis", these indicators may still have distinct biological mechanisms or medical consequences, which contribute to their prognostic significance in multivariate analysis.

We also discovered that a large tumor burden, in addition to the use of antibiotics and a high NLR, is a prognostic factor for poor OS. This observation is consistent with previous studies of ESCC that focused on patients with early-stage diseases undergoing surgery (24) or patients with recurrent or metastatic diseases receiving conventional chemotherapy (5, 25). This evidence, taken together, supports the use of tumor burden – the number of involved organ sites, for instance – as a stratification factor in large randomized trials of patients with ESCC. Besides, Huang et al., by investigating a group of melanoma patients treated with a PD-1 targeting antibody, found that the reinvigoration of exhausted-phonotype CD8+T cells was offset by a high tumor burden in patients who failed to derive benefit from PD-1 targeting therapy (26). Our observation may support this interaction between T-cell reinvigoration and tumor burden; however, the molecular mechanisms of this interaction and the clinical application of this observation warrant further research.

Our study has several limitations. First, our patient cohort was small, comprising only 49 patients, and their treatments were not homogeneous. Nevertheless, all our patients received PD-1/PD-L1-blockade-based therapy, but no additional conventional therapies such as chemotherapy or targeted therapy. No concomitant use of other potentially active agents in our study makes our observations relevant to the efficacy of PD-1/PD-L1–blockade immunotherapy. Second, the use of antibiotics was associated with variable clinical conditions, and the duration of antibiotics use was highly varied. Because of the limited number of patients in the cohort, we were not able to analyze the impacts of the clinical conditions that lead to the use of antibiotics, such as infection *versus* surgical prophylaxis, the duration of antibiotics treatment, or the timing of antibiotics use in relation to the start of immunotherapy. Lastly, we did not investigate the gut microbiome of our patients and thus could not address the hypothesis that the use of antibiotics changes the gut microbiome to contribute to the poor outcomes of our patients.

In summary, our study demonstrated that both a high NLR and the use of antibiotics were associated with poor prognoses in patients with recurrent or metastatic ESCC who received PD-1/PD-L1-blockade-based therapy. This observation warrants confirmatory studies using larger patient cohorts.

Conflicts of Interest

Dr. Chih-Hung Hsu served as an advisory role and received honoraria from Bristol-Myers Squibb, Ono Pharmaceutical, Merck Sharp & Dohme (MSD), and Genentech, and received research funding support from MSD. All other Authors declared no conflict of interest.

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Authors' Contributions

JCG, TCH and CHH designed the study, analyzed the data, and prepared the manuscript. All Authors acquired the data and reviewed the manuscript.

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