

# Patient background and laboratory variables associated with the efficacy of nivolumab after chemoradiotherapy in esophageal squamous cell carcinoma

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Received March 21, 2025; Accepted June 24, 2025

DOI: 10.3892/mco.2025.2885

**Abstract.** Esophageal squamous cell carcinoma (ESCC) is refractory to multidisciplinary treatment. Immunotherapy targeting programmed cell death protein 1 has shown promise in recent years; however, predicting its therapeutic efficacy remains difficult. The present study aimed to identify the factors associated with treatment response in patients receiving nivolumab after radical chemoradiotherapy for ESCC and to predict treatment efficacy based on these factors. A total of 22 patients were classified into partial response (PR) + stable disease (SD) and progressive disease (PD) groups based on their response to nivolumab. Factors associated with the treatment response were analyzed, including clinical stage, patient background and pre-treatment blood test results. Among the 22 patients, nine and 13 were classified into the PR + SD and PD groups, respectively. Notably, treatment efficacy had a significant impact on prognosis. Variables such as height, weight, body mass index, white blood cell count, basophil fraction, platelet count, albumin, creatinine and C-reactive protein were also evaluated. Cut-off values were determined using the Youden index for body weight (53.5 kg), white blood cells ( $5,000/\text{mm}^3$ ), basophils (0.6%), platelets ( $145 \times 10^4/\text{mm}^3$ ), albumin (3.9 mg/dl) and C-reactive protein (0.10 mg/dl). The number of favorable variables was calculated for each patient based on these cut-off values. Nine patients in the PR + SD group had three or more favorable variables, whereas 13 patients in the PD group had two or fewer. In conclusion, although the current study had a limited sample size, the six identified variables appear promising for predicting the efficacy of immune checkpoint inhibitors.

## Introduction

Esophageal cancer is the eighth most common cancer worldwide and the sixth leading cause of cancer-related mortality. Even in cases where the tumor is superficial, it exhibits a high metastatic potential with poor disease prognosis (1). Esophageal squamous cell carcinoma (ESCC) is the predominant type (2). ESCC remains refractory to multimodal treatment, with a 5-year survival rate of 20% in patients with stage IVb disease who are ineligible for surgery (3). Although neoadjuvant or definitive chemoradiotherapy (CRT) is the standard treatment for locally advanced ESCC, patient outcomes remain unsatisfactory, with recurrence rates as high as 30-50% (4). Recent studies have suggested a synergistic effect between CRT and immune checkpoint inhibitors (ICIs), highlighting the need for reliable biomarkers to predict the tumor response and survival outcomes (4).

Immunotherapy with ICIs has shown promise. However, the response rate to nivolumab, an anti-programmed cell death protein 1 (PD-1) antibody targeting the immunosuppressive auxiliary signal PD-1/PD-1 ligand 1 (PD-L1), in previously treatment-refractory ESCC was 19% in the ATTRACTION-3 trial (5). The CheckMate 648 trial (ClinicalTrials.gov, NCT03143153) (6) and KEYNOTE 590 trial (ClinicalTrials.gov, NCT03189719) (7) also demonstrated a certain degree of therapeutic efficacy, underscoring the urgent need to enhance immunotherapy response rates in ESCC.

Several studies have reported correlations between tumor immunity and inflammation, and the therapeutic effects of chemotherapy and immunotherapy. Nutrition-related factors, such as the prognostic nutritional index (PNI), which is based on serum albumin and lymphocytes (8,9), modified Glasgow prognostic score (mGPS), calculated from albumin and C-reactive protein (CRP) (10), CRP-to-albumin ratio (CAR) using albumin and lymphocytes (11), and controlling nutritional status (CONUT), which incorporates albumin, lymphocytes and total cholesterol (12,13), are widely used as prognostic indicators in cancers, including ESCC. These indices highlight the prognostic importance of albumin and C-reactive protein.

Predicting treatment effects is difficult; however, several reports have identified factors associated with treatment

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**Key words:** esophageal squamous cell carcinoma, nivolumab, chemoradiotherapy, immune checkpoint inhibitor

outcomes. Makino *et al* reported objective response and disease control rates of 17.0 and 47.9%, respectively, for nivolumab monotherapy in patients with unresectable advanced ESCC. They identified performance status, number of metastatic organs, CAR, neutrophil-lymphocyte ratio (NLR), and the psoas muscle index (PMI) as relevant influencing factors (14). Kosumi *et al* found that the waist circumference was significantly associated with the disease control rate at the initial evaluation and the PNI (15). These reports suggest that systemic factors, including the nutritional and cancer immunity status, influence the therapeutic efficacy of ICIs.

However, there are few reports on the factors that influence the therapeutic efficacy of nivolumab after definitive CRT. Given the availability of alternative therapies, such as paclitaxel, predicting nivolumab's efficacy is crucial, and a comprehensive evaluation, including assessment of systemic conditions and immune status, is needed.

Therefore, this study aimed to identify the factors influencing the therapeutic efficacy of nivolumab after CRT in ESCC and examine the prediction methods for treatment success.

## Materials and methods

**Patients.** We retrospectively analyzed patients with ESCC who received CRT following anti-PD-1 therapy (nivolumab administration) at Chiba University Hospital between September 2019 and February 2023. For comparison, the non-nivolumab group included patients who underwent radical CRT between January and December 2018, followed by chemotherapy other than nivolumab.

Patients were eligible for this study if they met the following criteria: i) A pathological diagnosis of ESCC; ii) age between 20-90 years, with the capacity for self-determination; iii) treatment with 5-fluorouracil + cisplatin (FP) as the sole chemotherapy regimen prior to nivolumab administration; and iv) all patients underwent blood tests after a sufficient interval following the completion of CRT or chemotherapy. Blood tests were performed on the day of nivolumab administration in 21 patients, and 7 days prior to administration in one patient.

The data of 22 patients who received nivolumab and 14 patients who did not receive nivolumab were retrospectively analyzed after excluding those who did not meet the inclusion criteria. Informed consent was obtained from all participants. Stage classification was performed according to the 11th edition of the Japanese Classification of Esophageal Cancer (16).

**Determination of treatment effectiveness.** Initial treatment effectiveness was determined using computed tomography after two or four courses of nivolumab. Patients were classified into two groups: the response group with partial response (PR) or stable disease (SD) and the non-response group with progressive disease (PD). Factors correlating with the treatment response were identified and analyzed.

**Blood test analysis.** Results of blood tests performed on or immediately before the day of nivolumab administration were analyzed. Differences in the following parameters between the PR + SD group and PD group were examined using the unpaired Student's t-test: white blood cell count (WBC, /all),

Table I. Patient characteristics (n=22).

| Characteristic                         | Value        |
|----------------------------------------|--------------|
| Median age (min-max), years            | 76 (52-89)   |
| Sex, Male: Female                      | 19:3         |
| Clinical T factor, T0/T1/T2/T3/T4a/T4b | 1/0/2/5/2/12 |
| Clinical N factor, N0/N1/N2/N3/N4      | 1/4/7/7/3    |
| Clinical M factor, M0/M1               | 18/4         |
| Clinical stage, II/III/IVa/IVb         | 3/3/12/4     |
| CPS, <1 /1-<10/≥10/NA                  | 0/4/8/10     |
| TPS %, <1/1-<10/≥10/NA                 | 3/2/7/10     |
| Effect of nivolumab, PR/SD/PD          | 5/4/13       |

Tumors were evaluated according to the Japanese Classification of Esophageal Cancer (11th edition). CPS, Combined Positive Score; TPS, Tumor Proportion Score.

leukocyte fraction (neutrophil, eosinophil, basophil, monocyte, and lymphocyte, %), hemoglobin (HGB, g/dl), platelet count (PLT,  $\times 10^3$ /all), total protein (TP, g/dl), albumin (ALB, g/dl), aspartate aminotransferase (AST, U/l), alanine aminotransferase (ALT, U/l), lactate dehydrogenase (LDH, U/l), bilirubin (BIL, mg/dl), creatinine (CRE, mg/dl), estimated glomerular filtration rate (eGFR, ml/min/1.73 m<sup>2</sup>), creatinine clearance in Cockcroft-Gault (CoCr, ml/min), and C-reactive protein (CRP, mg/dl). NLR, PNI, and CAR were also calculated and analyzed.

**Evaluation of categorical variables.** The Youden index was used to determine the cut-off values for the six key variables [body weight (BW), WBC, basophil, PLT, ALB, and CRP]. The significance of these cut-off values was assessed using Fisher's exact test. Kaplan-Meier survival curves were examined for overall survival (OS) and compared using the log-rank test. A total score was calculated based on the number of favorable variables identified, and association of the total score with nivolumab efficacy and OS were examined. In addition, pairwise associations among the six dichotomized variables were assessed using Fisher's exact test to examine potential inter-variable relationships.

**Statistical analyses.** Associations between the pathological grade of chemotherapy and clinicopathological features (e.g., sex and tumor factors such as clinical T and clinical N factors) were evaluated using Fisher's exact test. Blood test results were evaluated using unpaired Student's t-test. The Youden index was used to establish cut-off values for the six key variables. All statistical analyses were conducted using JMP Pro version 18 software (SAS Institute Inc., Cary, NC, USA).  $P < 0.050$  was considered to indicate a statistically significant difference, while values  $P < 0.100$  were considered a trend.

## Results

**Patient background characteristics.** The baseline characteristics of the 22 patients included in this study are shown in Table I. Of these, nine patients were in the PR + SD group,

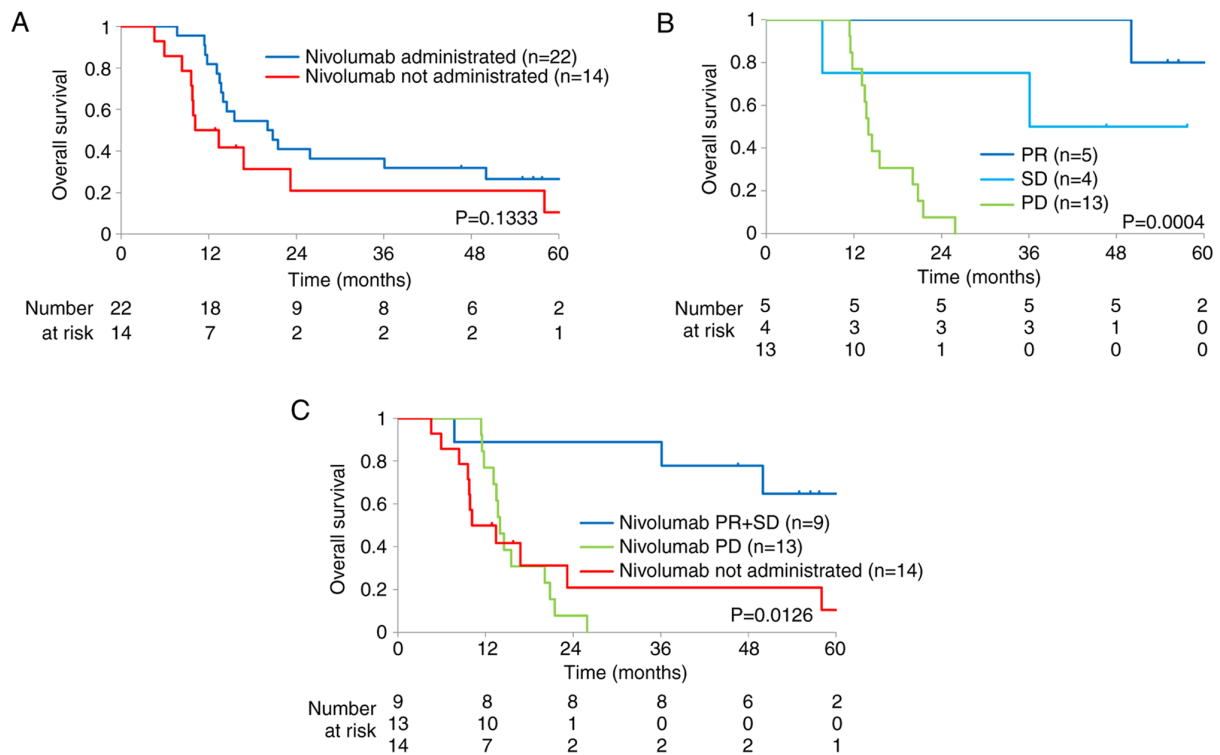


Figure 1. Association between nivolumab administration following CRT and OS. (A) OS of patients with or without nivolumab administration following CRT did not show a significant difference ( $P=0.1333$ ). (B) A large difference was observed in OS classified by the first treatment effect of nivolumab ( $P=0.0004$ ). (C) OS classified by the presence/absence of nivolumab treatment and first treatment effect was superior in the PR + SD group ( $P=0.0126$ ). CRT, chemoradiotherapy; OS, overall survival; PR, partial response; SD, stable disease; PD, progressive disease.

and 13 were in the PD group, with an overall response rate of 40.91%. There were no significant associations between age ( $P=0.6249$ ), sex ( $P=0.0002$ ), disease stage (tumor depth;  $P=1.0000$ , lymph node status;  $P=0.3602$ ), and PD-L1 expression [combined positive score (CPS);  $P=1.0000$ , tumor proportion score (TPS);  $P=1.0000$ ] in these two groups. PD-L1 expression was evaluated using both the CPS and TPS in 10 out of 22 patients. For the remaining 12 patients, PD-L1 data were not available. However, there were significant differences in weight ( $P<0.0001$ ), height ( $P=0.0231$ ), and body mass index (BMI) ( $P=0.0002$ ), related to the body size. Additionally, the number of nivolumab courses administered was significantly higher in the PR + SD group ( $P=0.0015$ ) (Table II). To investigate whether patient background characteristics differed by sex, we conducted the same analysis in only the 19 male patients (Table SI). The results showed a generally similar trend; however, the difference in body height was no longer statistically significant ( $P=0.1067$ ). When assessing OS, no significant difference was found between the nivolumab-treated and non-nivolumab-treated groups (Fig. 1A,  $P=0.1333$ ). In the nivolumab-treated group, there was a significant difference in prognosis when classified by the treatment effect, with a better prognosis in the PR + SD group and poorer prognosis in the PD group (Fig. 1B,  $P=0.0004$  and 1C,  $P=0.0126$ , respectively).

**Blood test analysis.** The association between blood test parameters and treatment response is summarized in Table III. WBC ( $P=0.0067$ ), basophil fraction ( $P=0.0042$ ), PLT ( $P=0.0229$ ), ALB ( $P=0.0388$ ), CRE ( $P=0.0196$ ), eGFR ( $P=0.0470$ ), and CRP ( $P=0.0939$ ) showed significant differences or trends

as determined by Student's t-test. Among the indices, CAR showed a trend toward a difference between the two groups ( $P=0.0926$ ); however, NLR and PNI did not show significant differences. Since reference ranges for blood test parameters can differ by sex, we reanalyzed the data by including only male patients (Table SII). Although the results showed a similar trend, both ALB ( $P=0.0692$ ) and CRP ( $P=0.1104$ ) did not reach statistical significance and demonstrated only a tendency.

**Classification and evaluation of BW and blood test results.** The classification and evaluation focused on variables showing significant differences: BW, WBC, basophil, PLT, ALB, and CRP. Given that no difference was observed in CoCr for renal function, CRE and eGFR were excluded from the evaluation because they were considered strongly influenced by the body size. The Youden index was used for determining the cut-off values for BW (53.5), WBC (5000), basophil (0.6), PLT (145), ALB (3.9), and CRP (0.10), dividing the patients into two groups. Fisher's exact test showed significant differences in BW ( $P=0.0005$ ), WBC ( $P=0.0075$ ), basophil ( $P=0.0048$ ), PLT ( $P=0.0274$ ), ALB ( $P=0.0260$ ), and CRP ( $P=0.0011$ ) (Table IV). Based on the results of Table SII, we also conducted this analysis in only male patients. In this subgroup, all six parameters showed statistically significant associations (Table SIII). Sex-based differences in these six variables were further examined (Table SIV). A significant difference was observed for BW ( $P=0.0015$ ), whereas no significant differences were found for the other blood test parameters. When associations between these categories and prognosis were

Table II. Relation between nivolumab efficacy and clinical characteristics.

| Characteristic         | Nivolumab efficacy  |                 | Disease control rate (%) | P-value |
|------------------------|---------------------|-----------------|--------------------------|---------|
|                        | PR + SD group (n=9) | PD group (n=13) |                          |         |
| Age, years             | 74.1±2.4            | 72.2±2.9        |                          | 0.6249  |
| Sex                    |                     |                 |                          |         |
| Male                   | 9                   | 10              | 47.4                     | 0.2403  |
| Female                 | 0                   | 3               | 0.0                      |         |
| Body weight, kg        | 62.7±2.0            | 47.5±1.9        |                          | <0.0001 |
| Body height, cm        | 166.6±1.5           | 159.0±2.7       |                          | 0.0231  |
| BMI, kg/m <sup>2</sup> | 22.56±0.56          | 18.77±0.58      |                          | 0.0002  |
| Tumor depth            |                     |                 |                          |         |
| T1/T2/T3/T4a           | 4                   | 6               | 40.0                     | 1.0000  |
| T4b                    | 5                   | 7               | 41.7                     |         |
| LN status              |                     |                 |                          |         |
| N0/N1                  | 1                   | 4               | 20.0                     | 0.3602  |
| N2/N3/N4               | 8                   | 9               | 47.1                     |         |
| Metastasis             |                     |                 |                          |         |
| M0                     | 8                   | 10              | 44.4                     | 0.6161  |
| M1                     | 1                   | 3               | 25.0                     |         |
| CPS                    |                     |                 |                          |         |
| <10                    | 2                   | 2               | 50.0                     | 1.0000  |
| ≥10                    | 4                   | 4               | 50.0                     |         |
| TPS %                  |                     |                 |                          |         |
| <10                    | 3                   | 2               | 60.0                     | 1.0000  |
| ≥10                    | 3                   | 4               | 42.9                     |         |
| Administrated courses  |                     |                 |                          |         |
| ≤4                     | 1                   | 12              | 7.7                      | 0.0015  |
| >4                     | 7                   | 2               | 77.8                     |         |
| Total                  | 9                   | 13              | 40.91                    | -       |

BMI, body mass index; LN, lymph node; CPS, Combined Positive Score; TPS, Tumor Proportion Score.

examined, favorable variables were associated with a better prognosis, with BW, WBC, basophil, and CRP demonstrating statistical significance (Fig. 2, log-rank: BW,  $P=0.0001$ ; WBC,  $P=0.0136$ ; basophil,  $P=0.0004$ ; PLT,  $P=0.1227$ , ALB,  $P=0.0777$ ; and CRP,  $P=0.0039$ ).

#### *Association between overall evaluation and prognosis.*

The number of favorable variables for each patient was determined based on the identified cut-off values. The total score was calculated for each patient based on the number of favorable variables within the following thresholds: body weight  $\geq 53.5$  kg, WBC  $\leq 5,000/\text{mm}^3$ , basophil  $\geq 0.6\%$ , platelet  $< 145 \times 10^4/\text{mm}^3$ , albumin  $\geq 3.9$  g/dl, and CRP  $\leq 0.10$  mg/dl. When assessing the associations among the categorized parameters (Table V), BW showed significant associations with the WBC ( $P=0.0300$ ), basophil count ( $P=0.0427$ ), platelet count ( $P=0.0396$ ), and CRP ( $P=0.0152$ ), but not with ALB. Additionally, WBC was significantly correlated with the basophil percentage ( $P=0.0351$ ). No other notable associations were observed among the remaining parameters.

Among all patients, nine from the PR + SD group had at least three favorable variables, whereas 13 from the PD group had two or fewer favorable variables (Table VI,  $P<0.001$ ). OS was significantly better in patients with three or more favorable variables than in those with two or fewer (Fig. 3, log-rank:  $P=0.0001$ ).

#### **Discussion**

In this study, we examined the association of patient background characteristics and blood parameters with nivolumab efficacy after CRT. We identified BW, WBC, percentage of basophil fraction, PLT, ALB, and CRP as the significant factors influencing nivolumab treatment after CRT.

The overall response and disease control rates with nivolumab treatment were 22.72 and 40.91%, substantially similar to those observed in the ATTRACTION-3 trial (5). In recent years, an abscopal effect has been associated with the post-CRT status in gastrointestinal cancers, such as esophageal, gastric, pancreatic, hepatobiliary, and colorectal

Table III. Association of blood test results with nivolumab efficacy.

| Item                                   | Nivolumab efficacy  |                 | P-value |
|----------------------------------------|---------------------|-----------------|---------|
|                                        | PR + SD group (n=9) | PD group (n=13) |         |
| WBC, /mm <sup>3</sup>                  | 4,522±267           | 6,346±528       | 0.0067  |
| Neutrophil, %                          | 68.87±1.90          | 71.98±1.78      | 0.2472  |
| Eosinophil, %                          | 2.10±0.62           | 2.14±0.51       | 0.9624  |
| Basophil, %                            | 0.71±0.08           | 0.38±0.06       | 0.0042  |
| Monocyte, %                            | 9.93±1.93           | 8.81±0.77       | 0.3937  |
| Lymphocyte, %                          | 18.39±1.51          | 16.69±1.46      | 0.4305  |
| HGB, g/dl                              | 11.21±0.61          | 10.90±0.51      | 0.7008  |
| PLT, x10 <sup>4</sup> /mm <sup>3</sup> | 178.0±19.1          | 256.8±25.6      | 0.0229  |
| TP, g/dl                               | 6.36±0.15           | 6.57±0.07       | 0.2264  |
| ALB, g/dl                              | 3.84±0.13           | 3.42±0.14       | 0.0388  |
| AST, IU/ml                             | 25.1±2.8            | 28.4±5.7        | 0.6116  |
| ALT, IU/ml                             | 17.6±4.1            | 30.3±9.8        | 0.2492  |
| LDH, IU/ml                             | 197.1±8.7           | 191.8±14.6      | 0.7567  |
| T-BIL, mg/dl                           | 1.07±0.49           | 0.53±0.05       | 0.3115  |
| CRE, mg/dl                             | 0.952±0.064         | 0.725±0.061     | 0.0196  |
| eGFR, ml/min/1.73 m <sup>2</sup>       | 63.02±6.23          | 82.78±6.95      | 0.0470  |
| CCr, ml/min                            | 64.13±7.60          | 63.54±5.34      | 0.9498  |
| CRP, mg/dl                             | 0.226±0.118         | 1.159±0.504     | 0.0939  |
| NLR                                    | 3.99±0.37           | 4.90±0.58       | 0.2027  |
| PNI                                    | 42.51±1.23          | 39.41±1.33      | 0.1029  |
| CAR                                    | 0.062±0.034         | 0.387±0.176     | 0.0926  |

Data are presented as mean ± standard error of the mean. WBC, white blood cell count; HGB, hemoglobin; PLT, platelet count; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; T-BIL, total bilirubin; eGFR, estimated glomerular filtration rate; CCr, creatinine clearance; CRP, C-reactive protein; NLR, neutrophil to lymphocyte ratio; PNI, prognostic nutritional index; CAR, CRP to albumin ratio.

cancers, (17). These findings, together with the results of this study, suggest that ICIs may be more selective after radiation therapy, especially in patients meeting the conditions indicated in this study.

BW and ALB are important indicators of the nutritional status. Although the PNI did not show a significant difference in this study, our findings underscore the importance of nutritional status. Previous studies in patients with non-small cell lung cancer have demonstrated that a higher BMI correlates with better prognosis in patients treated with ICIs, with BMI categorized as <18.5, 18.5-22.9, 23-24.9, and ≥25 (18). When considered alongside reports on waist circumference (15), it appears that body size, including body weight, is important in immunotherapy. However, additional studies are required to elucidate the underlying mechanism.

Interestingly, BW showed a significant association with several inflammation- and immunity-related parameters, including WBC, basophils, platelets, and CRP. These associations suggest that BW may reflect not only the long-term nutritional status but also systemic inflammatory and immune conditions. Given that WBC, basophils, and CRP are involved in tumor-associated immune modulation, BW could serve as an integrative marker of the host-tumor-immune axis.

Notably, in this analysis, BW did not show a significant association with ALB levels. While ALB is widely used as a nutritional marker, it is also a negative acute-phase reactant and can decrease rapidly in response to inflammation, due to cytokine-mediated suppression of hepatic synthesis (19). In contrast, BW may be less influenced by short-term inflammatory changes and could more accurately reflect the patient's baseline nutritional and immunological state. These findings warrant further investigation to determine whether BW functions as a surrogate marker or has a mechanistic role in the context of cancer-related inflammation and immunotherapy. WBC and CRP are direct inflammation-related factors. Cancers often induce both acute and chronic inflammation. In ESCC, high preoperative CRP levels have been associated with tumor progression and poor survival in patients (20). In environments with pre-existing systemic inflammation, tumor immunity may be impaired.

Among the WBC fractions, only basophils showed a significant difference; however, there are very few studies on the relationship between basophils and ICIs. In murine models, basophils have been shown to produce chemokines such as CCL3 and CCL4, thereby promoting the infiltration of CD8<sup>+</sup> T cells into the tumor microenvironment (21). This suggests that basophil activation may enhance antitumor

Table IV. Association of the classifications (BW, WBC, basophil, platelet, albumin, and CRP) and nivolumab efficacy.

| Item                                        | Nivolumab efficacy |               | Disease control rate (%) | P-value |
|---------------------------------------------|--------------------|---------------|--------------------------|---------|
|                                             | PR + SD group n=9  | PD group n=13 |                          |         |
| Body weight, kg                             |                    |               |                          |         |
| ≥53.5                                       | 9                  | 3             | 75.0                     | 0.0005  |
| <53.5                                       | 0                  | 10            | 0.0                      |         |
| WBC, /mm <sup>3</sup>                       |                    |               |                          |         |
| ≤5,000                                      | 8                  | 3             | 72.7                     | 0.0024  |
| >5,000                                      | 1                  | 10            | 9.1                      |         |
| Basophil, %                                 |                    |               |                          |         |
| ≥0.6                                        | 7                  | 3             | 70.0                     | 0.0113  |
| <0.6                                        | 2                  | 10            | 16.7                     |         |
| Platelet, x10 <sup>4</sup> /mm <sup>3</sup> |                    |               |                          |         |
| ≥145                                        | 4                  | 13            | 23.5                     | 0.0022  |
| <145                                        | 5                  | 0             | 100.0                    |         |
| Albumin, mg/dl                              |                    |               |                          |         |
| ≥3.9                                        | 6                  | 2             | 75.0                     | 0.0140  |
| < 3.9                                       | 3                  | 11            | 21.4                     |         |
| CRP, mg/dl                                  |                    |               |                          |         |
| ≤0.10                                       | 6                  | 0             | 100.0                    | 0.0006  |
| >0.10                                       | 3                  | 13            | 18.6                     |         |
| Total                                       | 9                  | 13            | 40.9                     | -       |

BW, body weight; WBC, white blood cell count; PLT, platelet count; ALB, albumin; CRP, C-reactive protein.

Table V. P-values of the pairwise associations among the six variables used to calculate the total score.

| Variable | BW | WBC    | Basophil | PLT    | CRP    | ALB    |
|----------|----|--------|----------|--------|--------|--------|
| BW       | -  | 0.0300 | 0.0427   | 0.0396 | 0.2043 | 0.0152 |
| WBC      |    | -      | 0.1984   | 0.0351 | 0.1827 | 0.1486 |
| Basophil |    |        | -        | 0.6241 | 0.3777 | 0.0557 |
| PLT      |    |        |          | -      | 0.3089 | 0.1005 |
| ALB      |    |        |          |        | -      | 0.1365 |
| CRP      |    |        |          |        |        | -      |

BW, body weight; WBC, white blood cell count; PLT, platelet count; ALB, albumin; CRP, C-reactive protein.

immunity by facilitating CD8<sup>+</sup> T cell-mediated tumor rejection. Furthermore, an increase in CCL3 levels within the tumor microenvironment was shown to promote T cell infiltration and enhance the efficacy of PD-1 blockade therapy (22). Although the mechanism remains unclear, one study suggested that basophils affect the tumor microenvironment via M2 macrophages (23), warranting further investigation. In patients with melanoma treated with anti-PD1 immunotherapy with a combination of nivolumab and ipilimumab, the relative basophil count was reported to correlate with the treatment response, along with relative eosinophils, absolute monocytes, LDH, and

NLR (24). Increased baseline basophil levels also predicted significantly improved progression-free survival (PFS) in patients with non-small cell lung carcinoma treated with nivolumab (25). In contrast, some studies have reported that patients with high basophil levels show poorer responses to ICI therapy. In gastric cancer, eosinophils have also been implicated in promoting M2 macrophage polarization, potentially contributing to an immunosuppressive microenvironment and resistance to immunotherapy (23). A recent mechanistic study revealed that IL-4 secreted by bone marrow-derived basophils drives the immunosuppressive phenotype of tumor-infiltrating monocyte-derived

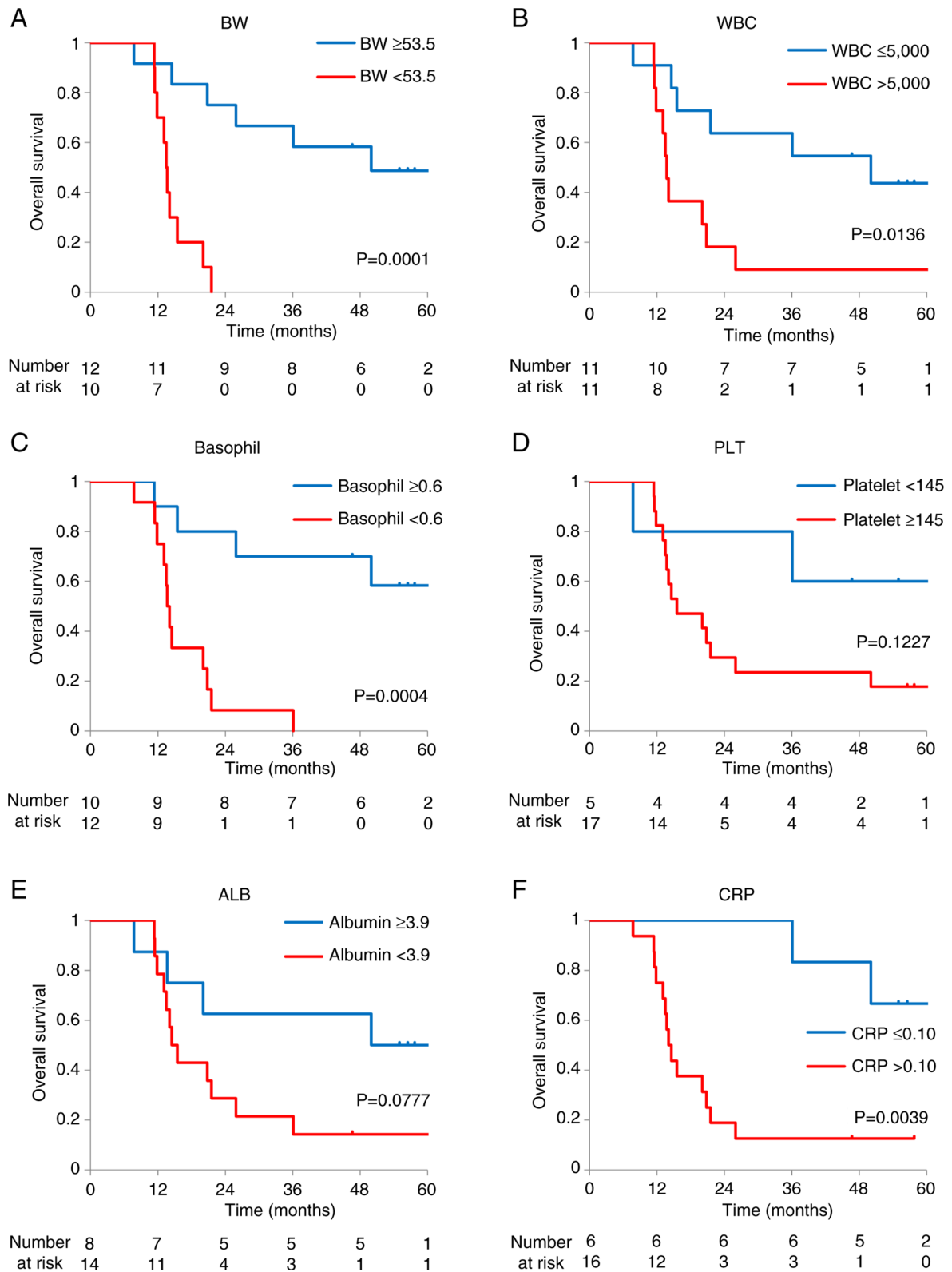


Figure 2. Association between categorical variables and OS. (A) Patients with body weight  $\geq 53.5$  kg had significantly better OS than those with body weight  $< 53.5$  kg (P=0.0001). (B) Patients with WBC  $\leq 5,000$  had significantly better OS than those with WBC  $> 5,000$  (P=0.0136). (C) Patients with basophil (%)  $\geq 0.6$  had significantly better OS than those with basophil (%)  $< 0.6$  (P=0.0004). (D) Patients with platelet  $< 145$  had better OS than those with relative platelet  $\geq 145$  (P=0.1227). (E) Patients with albumin  $\geq 3.9$  had better OS than those with relative albumin  $< 3.9$  (P=0.0777). (F) Patients with CRP  $\leq 0.10$  had significantly better OS than those with CRP  $> 0.10$  (P=0.0039). OS, overall survival; WBC, white blood cell count; CRP, C-reactive protein.

macrophages (26). This suggests that elevated basophil activity and IL-4 production may promote immune evasion and resistance to ICIs. These controversial results suggest

that differences between cancer types and interactions with other factors may need to be considered for a comprehensive evaluation.

Table VI. Association between total score and nivolumab efficacy.

| Total score | Nivolumab efficacy  |                 | Disease control rate (%) | P-value |
|-------------|---------------------|-----------------|--------------------------|---------|
|             | PR + SD group (n=9) | PD group (n=13) |                          |         |
| 0/1/2       | 0                   | 13              | 0.0                      | <0.0001 |
| 3/4/5/6     | 9                   | 0               | 100.0                    |         |

PD, progressive disease; PR, partial response; SD, stable disease.

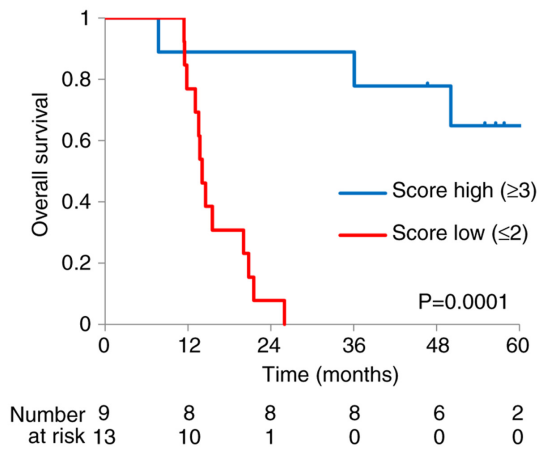


Figure 3. Association between total score and OS. Patients with total score  $\geq 3$  had significantly better OS than those with total score  $\leq 2$  ( $P=0.0001$ ). OS, overall survival.

In the present study, the cutoff value for basophils was set at 0.6% based on the Youden Index. Given that the normal reference range for basophils is approximately 0-1%, this cutoff was considered appropriate for dividing patients into high and low groups. However, further studies are warranted to validate the utility of this parameter.

PLTs act as active immune-like cells that suppress T cell activity, promote cancer metastasis, and regulate the spliced mRNA content (27). Inoue *et al* reported that nivolumab treatment for recurrent esophageal cancer was significantly more effective in patients with a low platelet-lymphocyte ratio, in addition to low lymphocytes and CAR (28). Additionally, Zhang *et al* reported that a lower systemic immune inflammation index ( $SII=PLT \times \text{neutrophils/lymphocytes}$ ) was associated with a significantly higher resection rate after preoperative treatment, including radiotherapy in ESCC (29). Although PLTs were often evaluated in combination with other parameters in previous studies, they were also identified as a significant factor in this study. Thus, it is now considered appropriate to evaluate the systemic inflammatory status not only by assessing the WBC count and CRP but also by combining various factors such as the lymphocyte fractions and PLT, which was also supported by the findings in this study.

In this study, CRE was significantly elevated, whereas the eGFR was lower in the PR + SD group. Furthermore, nivolumab did not require dose adjustment based on renal function, as antibodies are too large to be excreted by the

kidneys except in severe nonselective proteinuria (30). Given that body size parameters, such as height and weight, also showed significant differences, we evaluated renal function based on CoCr using the Cockcroft-Gault formula, which accounts for the BW. However, there was no significant difference in CoCr between the two groups, suggesting that the difference in creatinine clearance was due to differences in the body size.

In the present study, no significant association was found between PD-L1 expression and treatment efficacy. Moreover, data on other tumor-specific immunologic markers such as the tumor mutational burden were not available, and therefore not analyzed. These biomarkers are recognized as potentially important predictors of response to ICIs and should be investigated in future studies with a larger cohort.

The six variables identified in this study were considered valid when three or more variables were within the range in the PR + SD group. Based on this, we concluded that these variables may be useful for predicting the treatment efficacy.

However, this study has some limitations, including its small sample size and single-center design. The size of the cohort, consisting of 22 patients receiving nivolumab and 14 controls, limited the statistical power and may have introduced potential biases, thereby reducing the generalizability of the findings. Accordingly, the results should be interpreted with caution and regarded as hypothesis-generating rather than conclusive. Larger-scale, multicenter studies are needed to validate and extend these findings to broader clinical settings.

Although the scoring system using six variables demonstrated an apparent association with clinical outcomes, this should also be interpreted with caution given the exploratory nature of the study and limited sample size. Further validation in larger, independent cohorts will be required to confirm the predictive utility and clinical relevance of these parameters.

Moreover, according to the commonly used rule of thumb requiring at least 10 events per variable for logistic regression, including multiple variables in a multivariate model would have risked overfitting and unreliable estimates. Therefore, we limited our analysis to univariate methods. As the number of cases increases, the predictive value of these variables can be comprehensively examined, and the cut-off values will become more appropriate, enhancing the association between the number of applicable variables and treatment outcomes.

Finally, being a single-institution study, center-specific factors such as patient selection, treatment implementation, and healthcare infrastructure may have influenced the results. These findings should therefore be interpreted within the

context of this limitation, and future multicenter investigations will be essential to establish broader applicability.

In conclusion, although this study was based on a small sample of 22 cases, the six identified variables (BW, WBC, percentage of basophil fraction, PLT, ALB, and CRP) may be valuable for predicting the efficacy of ICI treatment after CRT in ESCC. By accumulating more cases based on our study's methodology, more reliable prediction methods can be developed.

## Acknowledgements

Not applicable.

## Funding

This study was supported by the Surgery II Scholarship Donation from the Department of Frontier Surgery, Graduate School of Medicine, Chiba University (grant no. J09KF00261).

## Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

## Authors' contributions

NS, TT, KM and HM contributed to the conceptualization of the study. NS, TT, KM, MU, AN, TS, KH, YM, YK, RO, SH and HH participated in data collection. NS, TT, KM, MU, AN and TS conducted project administration. NS and TT confirm the authenticity of all the raw data. NS wrote the manuscript under the supervision of TT, KM and HM. All authors provided critical feedback, and read and approved the final manuscript.

## Ethics approval and consent to participate

All procedures followed were in accordance with the Ethics Committee of Graduate School of Medicine, Chiba University (approval no. 3043; Chiba, Japan) and with the Helsinki Declaration of 1964 and later versions. Written informed consent was obtained from all patients before inclusion in the study.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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