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Pretreatment prognostic nutritional index as a novel biomarker in non-small cell lung cancer patients treated with immune checkpoint inhibitors

Fumihiro Shoji[a,*], Hiroaki Takeoka[b], Yuka Kozuma[a], Gouji Toyokawa[a], Koji Yamazaki[a], Masao Ichiki[b], Sadanori Takeo[a]

[a] Department of Thoracic Surgery, Clinical Research Institute, National Hospital Organization, Kyushu Medical Center, Japan
[b] Department of Respiratory Medicine, Clinical Research Institute, National Hospital Organization, Kyushu Medical Center, Japan

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ABSTRACT

Objectives: Immune checkpoint inhibitors (ICIs) have been established as a novel strategy for non-small cell lung cancer (NSCLC) therapy. However, a definitive biomarker that can predict response to ICI therapy remains unestablished. The prognostic nutritional index (PNI) is used to assess immune-nutritional conditions and is a prognostic factor in patients with various malignancies; however, its usefulness as a biomarker of response to ICI therapy and survival outcomes in NSCLC patients is unknown. Thus, we retrospectively analyzed the clinicopathological features of advanced-stage or recurrent NSCLC patients treated with ICI therapy to identify predictors of response to ICI therapy and investigate the effects of pretreatment PNI levels on survival after ICI therapy.

Materials and methods: We selected 102 consecutive NSCLC patients who were treated with ICI therapy from November 2015 to February 2019. We measured their pretreatment PNI levels and performed univariate and multivariate Cox regression analyses of progression-free survival (PFS) or overall survival (OS) after ICI therapy.

Results: Pretreatment PNI levels were significantly associated with response to ICI therapy (objective response rate:$P = 0.0131$; disease control rate: $P = 0.0002$), PFS ($P = 0.0013$), and OS ($P = 0.0053$). In univariate and multivariate analyses of the associations between PNI, C-reactive protein (CRP) or neutrophil-lymphocyte ratio (NLR) and PFS or OS, NLR and PNI, but not CRP, are independent prognostic factors for PFS (NLR: relative risk [RR] = 1.655, 95% confidence interval [CI]: 1.012–2.743, $P = 0.0449$, PNI: RR = 1.704, 95% CI: 1.039–2.828, $P = 0.0346$). Only PNI showed a trend towards being an independent prognostic factor for OS (RR = 1.606, 95% CI: 0.952–2.745, $P = 0.0761$).

Conclusion: The pretreatment PNI has the potential to be a simple and novel predictive biomarker of ICI response in NSCLC patients and might help to identify patients who will obtain a survival benefit from ICI therapy.

1. Introduction

Lung cancer is the leading cause of death from cancer worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers, and most NSCLC patients have advanced disease. In addition, only approximately 30% of NSCLC patients can receive surgical resection; however, up to 20% of patients, even those with early-staged NSCLC, relapse and die after undergoing surgical resections [2].

Immunotherapy with immune checkpoint inhibitors (ICIs) is widely used to treat various malignancies, including NSCLC, and has revolutionized therapeutic approaches to cancer. Programmed death-ligand-1 (PD-L1) is an immune checkpoint protein expressed on tumor cells and tumor-infiltrating immune cells. PD-L1 can mediate anticancer immunosuppression by binding to its receptors programmed cell death-1 (PD-1) and B7-1 [3]. Anti-PD-1 antibodies, such as nivolumab and pembrolizumab, inhibit PD-1-mediated signaling by blocking PD-L1 binding to PD-1. Anti-PD-L1 antibodies, such as atezolizumab, block PD-L1 interactions with either PD-1 or B7-1. These agents enable T-cell activation and immune system recognition.

Generally, PD-1 inhibitors used as single agents produce a clinical response in only 20% of NSCLC patients [4,5]; therefore, patient selection may be required to improve survival outcomes and prevent immune-related adverse events and unnecessary medical costs. Although tumorous PD-L1 expression is a potential biomarker of ICI

* Corresponding author at: Department of Thoracic Surgery, Clinical Research Institute, National Hospital Organization, Kyushu Medical Center, 1-8-1 Jigyohama, Chuo-ku, Fukuoka 810-8563, Japan.
E-mail address: fshoji@surg2.med.kyushu-u.ac.jp (F. Shoji).

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therapeutic response, it remains controversial whether it is truly the optimal predictor. Tumorous PD-L1 expression has been approved as a companion diagnostic test for pembrolizumab and high expression is associated with improved survival [6]; however, the role of tumorous PD-L1 expression remains controversial for nivolumab. Moreover, the Oak trial [7] demonstrated that NSCLC patients treated with atezolizumab obtained survival benefits regardless of PD-L1 expression level. In addition, recent studies (KEYNOTE-189 and -407, and IMpower-150) showed that ICIs such as pembrolizumab or atezolizumab combined with platinum-doublet chemotherapy yielded significantly longer overall survival (OS) and progression-free survival (PFS) in NSCLC patients with or without PD-L1 expression [8–10]. Thus, ICI response and survival outcomes show heterogeneity in NSCLC patients treated with ICIs.

The progression of tumors, including lung cancer, is closely associated with cancer-related inflammation and nutrition [11]. Several parameters have been used as objective tools for assessing the immune-nutritional status in lung cancer patients, and some have been identified as prognostic markers [12–15]. Among these tools, the prognostic nutritional index (PNI), which is based on serum albumin concentrations and total lymphocyte counts in the peripheral blood, was originally proposed to assess perioperative immune-nutritional status and surgical risk in patients undergoing gastrointestinal surgery [16]. We recently reported that the PNI was a prognostic marker for NSCLC [12]. However, the relationship between ICI response and the PNI remains uncertain.

Therefore, we hypothesized that the PNI plays a role as a predictive and prognostic factor in lung cancer treated with ICI therapy. This study retrospectively analyzed the clinicopathological features of NSCLC patients treated with ICI therapy to identify predictors of ICI therapy. We also investigated the association between pretreatment PNI and survival after ICI therapy.

2. Patients and methods

2.1. Patients

From November 2015 to February 2019, 102 consecutive patients with NSCLC treated with ICIs, including nivolumab (Opdivo, Bristol-Myers Squibb), pembrolizumab (Keytruda, Merck), and atezolizumab (Tecentriq, Genentech), at the Department of Thoracic Surgery and Department of Respiratory Medicine, Clinical Research Institute, Kyushu Medical Center were enrolled in the study. The center's institutional review board approved the study. The enrolled patients had at least one measurable target lesion based on the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 [17]. We excluded patients who had clinical evidence of dehydration, viral or bacterial infection, or other inflammatory conditions, as evaluated by the patients' symptoms, hematological and urinary tests, and imaging results (e.g., X-ray or computed tomography [CT]). We also excluded patients with hematological diseases and those who were currently taking drugs that might influence the hematological parameters measured in this study. Table 1 shows the patients' clinicopathological profiles. The results were determined in follow-up examinations over a median period of 201 days (range: 11–1064 months) after initial ICI therapy. The study group included 29 women and 73 men, with a mean age at the time of surgery of 69 years (range: 42–86 years). Sixty-six patients (64.7%) had ECOG performance status (PS) 0, 34 (33.3%) had ECOG-PS 1, and two (2.0%) had ECOG-PS 2. Thirty-one patients (30.4%) had never smoked, and the remaining 71 were current or former smokers. Their histological types were adenocarcinoma in 73 patients (71.6%), squamous cell carcinoma in 25 patients (24.5%), and other types in 4 patients (3.9%). Pathological stage was based on the Tumor Node Metastasis (TNM) classification of the International Union Against Cancer [18]. For TNM staging, all patients underwent CT of the thorax and upper abdomen, bone scintigrams, and brain CTs, magnetic resonance imaging (MRI), or fluorodeoxyglucose-positron emission tomography (FDG-PET). Of the 102 patients, 15 (14.7%) had stage III (2 with IIIA, 7 with IIIB, and 6 with IIIC), 58 (56.9%) had stage IV (48 with IVA and 10 with IVB), and 29 (28.4%) had postoperative recurrence. Twenty-seven patients (26.5%) had pulmonary metastases, 30 (29.4%) had bone metastases, 5 (4.9%) had liver metastases, 18 (17.6%) had brain metastases, and 5 (4.9%) had adrenal gland metastases. Twenty patients (19.6%) were treated as first-line therapy, 41 (40.2%) as second-line therapy, 20 (19.6%) as third-line therapy, and 21 (20.6%) as fourth-line or more therapy. Ninety-nine patients (98.0%) received ICIs as monotherapy (nivolumab: 3 mg/kg or 240 mg/body intravenously every 2 weeks; pembrolizumab: 200 mg/body intravenously every 3 weeks; or atezolizumab: 1200 mg/body intravenously every 3 weeks), and the remaining 3 patients received ICIs combined with platinum-doublet

Table 1
Clinicopathological profiles.

	No. (%) or Median (Range)
Total assessable patients	102 (100)
Follow-up period (day)	221, 11–1064
Age, years	69, 42–86
Sex	
Female	29 (28.4)
Male	73 (71.6)
ECOG performance status	
0	66 (64.7)
1	34 (33.3)
2	2 (2.0)
Smoking status	
Never	31 (30.4)
Current/former	71 (69.6)
Histology	
Adenocarcinoma	73 (71.6)
Squamous cell carcinoma	25 (24.5)
Others	4 (3.9)
Tumor stage	
III (IIIA/IIIB/IIIC)	15 (2/7/6) (14.7)
IV (IVA/IVB)	58 (48/10) (56.9)
Postoperative Recurrence	29 (28.4)
Distant metastatic sites	
Lung	27 (26.5)
Bone	30 (29.4)
Liver	5 (4.9)
Brain	18 (17.6)
Adrenal gland	5 (4.9)
Lines of prior therapy	
0	20 (19.6)
1	41 (40.2)
2	20 (19.6)
≥3	21 (20.6)
Regimen	
Nivolumab monotherapy	63 (61.8)
Pembrolizumab monotherapy	24 (23.5)
Atezolizumab monotherapy	12 (11.7)
CBDCA/PEM/Pembrolizumab	2 (2.0)
CBDCA/nab-Pac/Pembrolizumab	1 (1.0)
Targetable driver mutation	
EGFR	16 (19.0)
ALK	1 (1.4)
ROS1	0 (0.0)
PD-L1 expression	
Tumor proportion score (%)	
≥50	21 (20.6)
1-49	19 (18.6)
0	8 (7.9)
Not evaluated	54 (52.9)
Prognostic nutritional index	
High	50 (49.0)
Low	52 (51.0)

EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, proto-oncogene tyrosine kinase; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PD-L1, programmed death-ligand-1.

chemotherapy (pembrolizumab: 200 mg/body plus carboplatin; area under the curve [AUC] for concentration-time: 5 mg per milliliter per minute plus pemetrexed 500 mg per square meter or nab-paclitaxel 200 mg per square meter intravenously every 3 weeks). ICI therapy was continued until radiographic progression or unacceptable adverse events (AEs). AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Sixteen patients (19.0%) had mutant epidermal growth factor receptor, and one (1.4%) had rearrangement of anaplastic lymphoma kinase. We evaluated the PD-L1 protein expression using antibody clone 22C3 (Dako, Agilent Technologies, Santa Clara, CA, USA). Twenty-one patients (20.6%) had more than 50% tumorous PD-L1 expression, 19 (18.6%) had 1–49% PD-L1 expression, and 8 (7.9%) had no PD-L1 expression. Postoperative local or distant recurrence was defined as described in a previous report [19]; specifically, local recurrence was defined as that occurring in the hilar or mediastinal lymph nodes, pleural cavity, bronchial stump, or staple line; and all other sites, including the brain, lung, adrenal glands, and bone, were considered distant recurrences. The first appearance of any new lesion suspected to be a recurrence of the original lung cancer was defined as postoperative recurrence and was clinically diagnosed by combinations of CT, MRI, bone scintigram, and FDG-PET, or was pathologically diagnosed if necessary.

2.2. Pretreatment calculation of the PNI and the PNI cut-off value

The pretreatment PNI was calculated using the following formula: 10 × serum albumin level (g/dl) + 0.005 × total lymphocyte count in the peripheral blood (per mm^3) within 7 days immediately preceding ICI treatment. The receiver operating characteristic (ROC) curve of the pretreatment PNI levels was analyzed, and ICI response was predicted by comparing the AUC. We determined that the best cut-off value for the pretreatment PNI was 45.5 (sensitivity: 68.0%; specificity: 70.0%; AUC of the ROC curve: 0.694). Fifty patients (49.0%) had pretreatment PNI levels > 45.5 (high-PNI) and the remaining 52 (51.0%) patients had pretreatment PNI ≤ 45.5 (low-PNI) (Table 1). The best cut-off value for pretreatment C-reactive protein (CRP) concentration (mg/dl) was 0.51 (sensitivity: 54.0%; specificity: 71.2%; AUC of the ROC curve: 0.622) and for the neutrophil-lymphocyte ratio (NLR = absolute neutrophil count divided by absolute lymphocyte count) was 3.88 (sensitivity: 66.0%; specificity: 63.5%; AUC of the ROC curve: 0.643).

2.3. Statistical analysis

Categorical variables were analyzed using Fisher's exact test. Continuous variables are expressed as the mean and standard error (SE), and means were compared using the chi-square test. PFS was defined as the interval between initial ICI treatment and the time of diagnosis of progressive disease (PD). Overall survival (OS) was defined as the time between initial ICI treatment and death from any cause. We analyzed patient survival using the Kaplan–Meier method and compared groups using the log-rank test. Univariate and multivariate analyses were performed using a logistic regression model. A Cox proportional hazards model was used to identify independent predictive and prognostic factors. $P < 0.05$ was considered significant. All statistical analyses were performed using JMP software, version 14.0 (SAS Institute, Inc.).

3. Results

3.1. Association between pretreatment PNI level and ICI response

Supplementary Fig. 1 shows the distribution of pretreatment PNI values for patients with partial response (PR)/stable disease (SD), and PD. Pretreatment PNI levels were significantly lower in patients with PD than in those with PR/SD (mean ± SE, PD patients 41.99 ± 0.95 vs. PR/SD patients 46.60 ± 0.97, $P = 0.0010$). **Supplementary Table 1** shows the ICI response based on pretreatment PNI levels. Of the 50 patients with high-PNI pretreatment levels, 13 (26.0%) experienced PR, 21 (42.0%) experienced SD, and 16 (32.0%) experienced PD. Of the 52 patients with low-PNI pretreatment levels, 4 (7.7%) experienced PR, 12 (23.1%) experienced SD, and 36 (69.2%) experienced PD. The two groups differed significantly in their response to ICI therapy ($P = 0.0006$). The associations between pretreatment PNI level and objective response rate (ORR) were statistically significant, with ORRs of 7.69% for the low-PNI group and 26.0% for the high-PNI group (odds ratio [OR]: 4.22, 95% confidential interval [CI]: 1.28–14.00), $P = 0.0131$) (Table 2). The associations between pretreatment PNI level and disease control rate (DCR) were also statistically significant, with DCRs of 32.0% for the low-PNI group and 68.0% for the high-PNI group (OR: 4.78, 95% CI: 2.07–11.04, $P = 0.0002$) (Table 2). In parallel, we analyzed the association between clinical efficacy and pretreatment serum lymphocyte count or albumin level separately. For this, we used the "under-immune-nutrition" serum lymphocyte count (1200/mm^3) and albumin concentration (3.0 g/dl) defined by Ignacio de Ulíbarri et al. [20]. The analysis revealed no significant associations between either the serum lymphocyte count (< 1200/mm^3 vs. ≥ 1200/mm^3) or albumin concentration (< 3.0 g/dl vs. ≥ 3.0 g/dl) and either ORR (lymphocyte count OR: 1.01, 95% CI: 0.46–2.21, $P = 0.7894$; albumin concentration OR: 2.63, 95% CI: 0.32–21.65, $P = 0.9813$) or DCR (lymphocyte count OR: 0.87, 95% CI: 0.31–2.46, $P = 0.3526$; albumin concentration OR: 2.41, 95% CI: 0.69–8.39, $P = 0.1588$) (Table 2).

Table 2
Association between pretreatment clinical response and serum lymphocyte count, albumin concentration, and prognostic nutritional index (PNI) for non-small cell lung cancer patients treated with immune checkpoint inhibitors.

	Rate	ORR OR (95% CI)	Rate	DCR OR (95% CI)
Low-lymphocytes count (< 1,200/mm^3)	17.8%	1 (reference)	48.9%	1 (reference)
High-lymphocytes count (≥ 1,200/mm^3)	15.8%	0.87 (0.31–2.46)	49.1%	1.01 (0.46–2.21)
Low-albumin concentration (< 3.0 g/dl)	7.69%	1 (reference)	30.8%	1 (reference)
High-albumin concentration (≥ 3.0 g/dl)	18.0%	2.63 (0.32–21.65)	51.7%	2.41 (0.69–8.39)
Low-PNI level (< 45.5)	7.69%	1 (reference)	32.0%	1 (reference)
High-PNI level (≥ 45.5)	26.0%	4.22 (1.28–14.00) *	68.0%	4.78 (2.07–11.04) **

CI, confidence interval; DCR, disease control rate; OR, odds ratio; ORR, objective response rate. *$P = 0.0131$, ** $P = 0.0002$.

3.2. Immune-related AEs

Eleven patients (10.8%) experienced immune-related AEs of any grade. **Supplementary Table 2** summarizes the frequencies and types experienced. The most frequent immune-related AE was interstitial pneumonia.

3.3. Prognostic factors in NSCLC patients treated with ICI therapy

We compared PFS and OS in patients according to age (> 75 years vs. ≤ 75 years), sex (male vs. female), smoking status (former or current smokers vs. never-smokers), ECOG-PS (1–2 vs. 0), number of prior systemic therapies (0 vs. ≥ 1), regimen (monotherapy vs. combined therapy), histology (nonadenocarcinoma vs. adenocarcinoma), tumor stage (IV vs. III), tumor proportion score (TPS) > 50% PD-L1 expression (no vs. yes), TPS > 1% PD-L1 expression (no vs. yes), driver oncogenes

Table 3
Univariate and multivariate analyses of factors associated with progression-free survival in NSCLC patients treated with ICIs.

Variable	Univariate analysis RR (95% CI), *P* value	Multivariate analysis RR (95% CI), *P* value
Age (> 75 vs. ≤ 75)	0.870 (0.676- 2.073), 0.6190	–
Sex (male vs. female)	1.102 (0.676- 1.858), 0.7022	–
Smoking status (cur/for vs. never)	1.139 (0.698- 1.821), 0.5949	–
ECOG PS (1-2 vs. 0)	1.594 (0.987- 2.647), 0.0568	–
No. of prior systemic therapy (0 vs. ≥ 1)	0.566 (0.272- 1.059), 0.0767	–
Regimen (mono vs. combined therapy)	1.459 (0.540- 5.983), 0.4987	
Histology (non-Ad vs. Ad)	1.881 (1.111- 3.086), 0.0197	1.297 (0.727- 2.282), 0.3727
Tumor stage (IV vs. III)	2.019 (0.996- 4.659), 0.0513	–
TPS ≥50% (No vs. yes)	1.137 (0.551- 2.406), 0.7287	–
TPS ≥1% (No vs. yes)	1.516 (0.500- 3.809), 0.4303	–
Driver oncogenes (With vs. without)	0.908 (0.458- 1.657), 0.7633	–
CRP (High vs. low)	1.837 (1.144- 3.017), 0.0116	1.315 (0.758- 2.292), 0.3289
NLR level (High vs. low)	2.109 (1.327- 3.399), 0.0016	1.655 (1.012- 2.743), 0.0449
PNI level (Low vs. high)	2.135 (1.333- 3.462), 0.0016	1.704 (1.039- 2.828), 0.0346

Ad: adenocarcinoma; CI: confidence interval; Cur/for: current/former smoker; ICIs: immune checkpoint inhibitors; NSCLC: non-small cell lung cancer; PD-L1: programmed death ligand 1; PNI: prognostic nutritional index; PS: performance status; RR: relative risk; TPS: tumor proportion score.

Table 4
Univariate and multivariate analyses of factors associated with overall survival in NSCLC patients treated with ICIs.

Variable	Univariate analysis RR (95% CI), *P* value	Multivariate analysis RR (95% CI), *P* value
Age (> 75 vs. ≤ 75)	0.791 (0.411- 1.415), 0.4435	–
Sex (male vs. female)	1.394 (0.816- 2.484), 0.2295	–
Smoking status (cur/for vs. never)	1.247 (0.739- 2.177), 0.4153	–
ECOG PS (1-2 vs. 0)	1.836 (1.072- 3.296), 0.0262	1.932 (1.116- 3.506), 0.0180
No. of prior systemic therapy (0 vs. ≥ 1)	0.842 (0.402- 1.592), 0.6147	–
Regimen (mono vs. combined therapy)	2.302 (0.504- 40.763), 0.3425	
Histology (non-Ad vs. Ad)	2.719 (1.541- 4.723), 0.0007	2.308 (1.239- 4.305), 0.0087
Tumor stage (IV vs. III)	1.420 (0.671- 3.490), 0.3789	–
TPS ≥50% (No vs. yes)	1.211 (0.525- 2.818), 0.6507	–
TPS ≥1% (No vs. yes)	1.991 (0.553- 5.781), 0.2654	–
Driver Oncogenes (With vs. without)	1.009 (0.477- 1.931), 0.9803	–
CRP (High vs. low)	2.360 (1.391- 4.115), 0.0013	0.698 (0.373- 1.300), 0.2570
NLR level (High vs. low)	2.031 (1.232- 3.389), 0.0055	1.524 (0.895- 2.623), 0.1211
PNI level (Low vs. high)	2.022 (1.226- 3.383), 0.0063	1.606 (0.952- 2.745), 0.0761

Ad: adenocarcinoma; CI: confidence interval; Cur/for: current/former smoker; ICIs: immune check point inhibitors; NSCLC: non-small cell lung cancer; PNI: prognostic nutritional index; PS: performance status; RR: relative risk; TPS: tumor proportion score.

(present vs. absent), pretreatment CRP level (high vs. low), pretreatment NLR (high vs. low), and pretreatment PNI level (low vs. high) (Tables 3 and 4). Univariate analysis showed that histology ($P = 0.0197$), pretreatment CRP level ($P = 0.0116$), pretreatment NLR ($P = 0.0016$), and pretreatment PNI level ($P = 0.0016$) significantly affected PFS. The relative risks (RRs) were 1.881 for non-adenocarcinoma versus adenocarcinoma (95% CI: 1.111–3.086), 1.837 for high-CRP level versus low-CRP level (95% CI: 1.144–3.017), 2.109 for high-NLR versus low-NLR (95% CI: 1.327–3.399), and 2.135 for low-PNI level versus high-PNI level (95% CI: 1.333–3.462). In the multivariate analysis, NLR (RR: 1.655; 95% CI: 1.012–2.743; $P = 0.0449$) and PNI (RR: 1.704; 95% CI: 1.039–2.828; $P = 0.0346$) were independent prognostic factors for PFS (Table 3). Univariate analysis showed that ECOG-PS ($P = 0.0262$), histology ($P = 0.0007$), pretreatment CRP level ($P = 0.0013$), pretreatment NLR ($P = 0.0055$), and pretreatment PNI level ($P = 0.0063$) significantly affected OS. The RRs were 1.836 for ECOG-PS 1–2 versus ECOG-PS 0 (95% CI: 1.072–3.296), 2.719 for nonadenocarcinoma versus adenocarcinoma (95% CI: 1.541–4.723), 2.360 for high-CRP level versus low-CRP level (95% CI: 1.391–4.115), 2.031 for high-NLR versus low-NLR (95% CI: 1.232–3.389), and 2.022 for low-PNI level versus high-PNI level (95% CI: 1.226–3.383). In the multivariate analysis, ECOG-PS (RR: 1.932; 95% CI: 1.116–3.506; $P = 0.0180$) and histology (RR: 2.308; 95% CI: 1.239–4.305; $P = 0.0087$) were independent prognostic factors for OS. PNI showed a trend towards being a prognostic factor for OS but did not reach the level of statistical significance (RR: 1.606; 95% CI: 0.952–2.745; $P = 0.0761$) (Table 4).

3.4. Pretreatment PNI, PFS, and OS in advanced and recurrent NSCLC patients

Kaplan–Meier analysis of the PFS and OS for 102 patients with advanced or recurrent NSCLC stratified by PNI level showed that the low-PNI group had significantly shorter PFS and OS than did the high-PNI group (PFS; median survival time [MST]: low-PNI vs. high-PNI = 70 days vs. 169 days, $P = 0.0013$, log-rank test [Fig. 1] and OS; MST: low-PNI vs. high-PNI = 218 days vs. 524 days, $P = 0.0053$, log-rank test [Fig. 2]).

4. Discussion

The present study showed two novel findings. First, the pretreatment PNI level was significantly associated with ORR and DCR in NSCLC patients treated with ICIs, specifically, a low pretreatment PNI indicated a low response to ICI therapy. Thus, the pretreatment PNI has the potential to predict ICI response in NSCLC patients, although its usefulness as a biomarker will need to be verified by future prospective studies. Second, multivariate analysis showed that low pretreatment PNI and low pretreatment NLR were independent prognostic factors for PFS in NSCLC patients after ICI therapy, whereas only pretreatment PNI

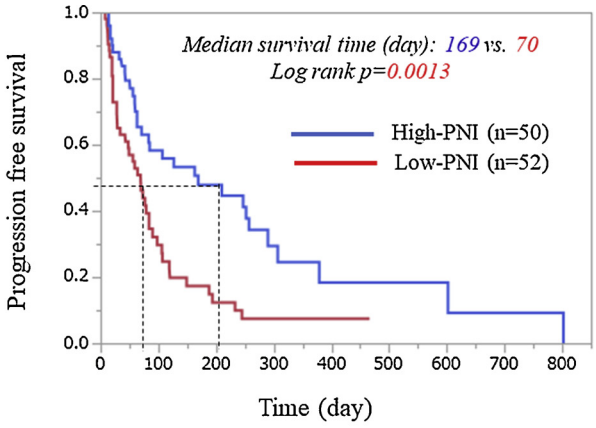

Fig. 1. Kaplan-Meier curve analysis of progression-free survival for 102 non-small cell lung cancer (NSCLC) patients treated with immune checkpoint inhibitor (ICI) therapy by pretreatment prognostic nutritional index (PNI) level. Blue line: high-PNI group; red line: low-PNI group. The two groups differed significantly (median survival time: 169 days vs. 70 days, $P = 0.0013$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Patient at risk

	0	100	200	300	400	500	600	700	800
High-PNI	50	25	16	7	4	4	3	2	2
Low-PNI	52	13	6	4	3	1	1	1	1

was a prognostic factor for OS. Diem et al. [21] reported that pretreatment NLR was a prognostic factor in NSCLC patients treated with nivolumab, and Oya et al. [22] demonstrated that CRP was associated with survival in patients treated with nivolumab. Therefore, we performed univariate and multivariate analyses of PFS and OS with these candidate biomarkers. **Supplementary Table 3** shows the comparison of AUC, sensitivity, and specificity of the three potential biomarkers (CRP, NLR, and PNI), and the results indicate that PNI was the more useful biomarker compared with CRP or NLR in this retrospective study. In addition, in the pretreatment low-PNI group, both MST of PFS and OS were significantly shorter than those in the pretreatment high-PNI group. Thus, PNI also has the potential to be a prognostic factor for NSCLC patients treated with ICI therapy.

Biomarkers such as PD-L1, tumor-infiltrating lymphocytes (TILs), and tumor mutation burden are candidate predictive factors for response to ICI therapy. Nevertheless, not all patients with these predictive factors benefit from immunotherapy. PD-L1 expression on tumor cells is a confirmed predictive factor for ICI therapy in NSCLC patients investigated in prospective clinical trials. However, the results of clinical trials investigating the role of PD-L1 expression in qualification for immunotherapy are conflicting, most likely for several reasons. First, the studies used different randomization methods according to the PD-L1 status on tumor and immune cells [4,5,23–27]. Second, the studies used different monoclonal antibody clones to evaluate PD-L1 expression by immunohistochemical staining [4,5,8–10,23–29]. Third, PD-L1 expression in tumors is known to be highly heterogeneous [30]. For example, McLaughlin et al. [31] reported that PD-L1 expression in NSCLC patients was heterogenous within different regions of the same tumor, which might also apply to TILs. Fourth, PD-L1 expression may have changed during previous treatments such as radiotherapy or chemotherapy [32]. Fifth, several clinical trials [4,5,25] showed that second-line treatment with anti-PD-1 or anti-PD-L1 antibodies showed efficacy even in patients lacking PD-L1 expression on tumor or immune cells. Conversely, many patients with PD-L1 expression on tumor or immune cells do not respond to immunotherapy [4,5,25]. These observations suggest that PD-L1 expression is insufficiently sensitive and/or specific for patient stratification, highlighting the need for new predictive factors that can be used to qualify NSCLC patients to immunotherapy. Patients with high PD-L1 expression on ≥50% of tumor cells clearly respond to first-line therapy

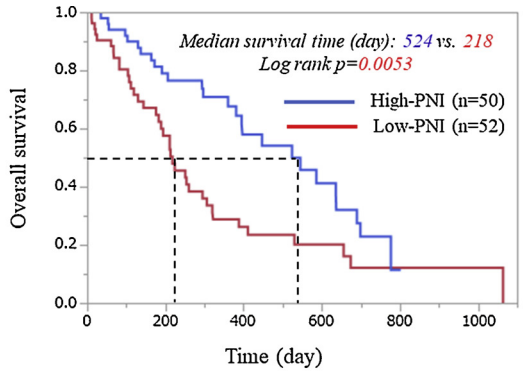

Fig. 2. Kaplan-Meier curve analysis of overall survival for 102 non-small cell lung cancer (NSCLC) patients treated with immune checkpoint inhibitor (ICI) therapy by pretreatment prognostic nutritional index (PNI) level. Blue line: high-PNI group; red line: low-PNI group. The two groups differed significantly (median survival time: 524 days vs. 218 days, $P = 0.0053$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Patient at risk

	0	100	200	300	400	500	600	700	800	900	1000	1100
High-PNI	50	46	34	26	19	14	10	6	2	1	1	1
Low-PNI	52	38	25	16	11	9	6	4	4	3	3	1

with pembrolizumab, but some patients with lower or even no expression of PD-L1 still respond and show extended PFS and OS with second-line ICI treatment [4,8–10,25,24–29]. These findings indicate that other factors may be superior to high PD-L1 expression as positive predictive factors for ICI therapy. PD-L1 expression on tumor cells or TILs is generally evaluated using archived samples from advanced or recurrent NSCLC patients, except in cases of first-line ICI therapy or when fresh samples were obtained via rebiopsy immediately before starting ICI therapy. Therefore, many investigators note that tumorous or TIL PD-L1 expression may not accurately reflect the patient's immune condition immediately prior to ICI therapy. Ideally, a fresh sample from the target lesions should be obtained by rebiopsy in all cases before starting ICI therapy, although this is not always practical.

In the present study, PD-L1 expression was examined in 48 of the 102 patients. Of the remaining 54 patients, 52 were treated with nivolumab monotherapy in a second-line setting, and 2 were treated with chemotherapy combined with ICIs in a first-line setting. We did not examine PD-L1 expression in these 54 patients based on the results of previous clinical trials, as described above. We note that our analyses of the significance of PD-L1 expression level only included data from the 48 patients whose tumor PD-L1 expression was assessed. As a result, tumorous PD-L1 expression did not correlate with response to ICI therapy or survival outcome after ICI therapy. However, the number of cases evaluated was limited, and multicentric and prospective studies will be needed to more clearly delineate the impact of PD-L1 expression on response to monotherapy or combination therapy with ICIs. Technical complexity is a potential complicating factor in measuring tumor mutation burden, such as the long turn-around times and large tumor volumes required to perform the analysis, and controversies also exist regarding data interpretation and cut-off values [33].

Hematological testing is simple and inexpensive and yields current information on immune status just prior to ICI therapy. Among several parameters based on hematological data, the PNI is a particularly attractive biomarker. Onodera et al. proposed the use of a modified PNI calculated from serum albumin concentration and lymphocyte counts in the peripheral blood [34]. Since then, many studies have reported the usefulness of PNI as a prognostic factor for various malignancies [35–41]. Lymphocytes play a fundamentally important role in anticancer immune responses, not only through their direct cytolytic activity but also through their ability to recruit and amplify the activity of other anti-tumor immune cells and to inhibit cancer cell proliferation, invasion, and migration [42,43]. Given that the anticancer immune response is mainly mediated by lymphocytes, the presence of lymphopenia might reflect a reduced ability to mount an anticancer response and a low lymphocyte count might thus be indicative of tumor progression. Serum albumin is the simplest and most valuable parameter for assessing nutritional status. Nutrition is an important determinant of host immune systems; therefore, albumin levels may reflect the degree of host immunity impairment [44]. The nutritional condition also plays important roles in the effectiveness of chemotherapy. Ross et al. [45] reported relationships between malnutrition status and poor response to cytotoxic chemotherapy and poor prognosis in lung cancer patients. Thus, nutrition is also thought to affect disease progression in cancer patients. Although PNI is a measure of both the immunological and nutritional condition of a patient, no studies had previously confirmed its usefulness as a predictive and prognostic factor in NSCLC patients treated with ICIs. The present study showed that pretreatment PNI levels were both a predictive factor of the response to ICI therapy and a prognostic factor for NSCLC patients after ICI therapy. A meta-analysis of studies of surgically resected gastric cancer patients identified cut-off values for PNI ranging from 45 to 49.7 [46], which is similar to the cut-off value used in the present study.

We also investigated whether pretreatment PNI levels predicted the duration of the ICI therapy **(Supplementary Fig. 2)**. This analysis showed that pretreatment PNI levels were significantly higher in patients with > 5 cycles compared with ≤ 4 cycles(mean ± SE: 45.95 ± 0.96 vs. 42.41 ± 1.0, $P = 0.0121$). Thus, high pretreatment PNI levels could also predict the requirement for multiple ICI therapy cycles.

Major advancements in microbiome research have occurred in recent years, and correlations between the microbiome, host immunity, and disease, including cancer, have been reported. Several studies showed that the commensal microbiome activated the host immune response and improved the efficacy of anti-PD-L1 antibody treatment [47–49]. Matson et al. [48] reported that fecal microbiome transplantation from ICI-responder patients to non-ICI-responder patients improved the efficacy of the response to ICI therapy via augmentation of the T-cell response. That study strongly supports our finding here that host immunity is associated with ICI therapy response and survival outcomes. We suggest that evaluation of the immune-nutritional status should be considered for settings in which ICIs are combined with chemotherapy as well as when chemoradiotherapy is followed by ICIs. We are currently planning a study to investigate whether the immune-nutritional condition is related to the oral or commensal microbiome in NSCLC patients, which may elucidate the detailed mechanisms of host immunity to ICI therapy.

Finally, this investigation was a single institutional and retrospective study, and a multicentric large-scale prospective study is warranted to evaluate the PNI criteria employed in this study, the predictive biomarkers of response to ICI therapy, and the survival benefit of ICI therapy for NSCLC patients according to pretreatment PNI levels. In addition, immune-nutritional support before or during ICI therapy could potentially improve the response and outcomes to ICI therapy in patients with advanced or recurrent disease. A prospective study to certify the usefulness of immune-nutritional support in NSCLC patients treated with ICI therapy is also needed.

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Declaration of Competing Interest

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.08.006.

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