Original Article

PDL-1 Expression and Survival in Metastatic Non-small Cell Lung Cancer Patients Who Received Chemotherapy as First-Line Treatment

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Abstract

OBJECTIVE: To show the effect of programmed cell death protein-1 ligand level survival times in patients with metastatic non-small cell lung cancer receiving chemotherapy, to determine the relationship between programmed cell death protein-1 ligand level, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio.

MATERIAL AND METHODS: The data of 158 patients who received chemotherapy for metastatic non-small cell lung cancer were evaluated retrospectively. Clinical and demographic data, programmed cell death protein-1 ligand expression levels, and follow-up periods of the patients were recorded. The patients were divided into 2 groups according to programmed cell death protein-1 ligand levels.

RESULTS: In all patients, progression-free survival was 5.6 months and overall survival was 18.8 months. Patients with low programmed cell death protein-1 ligand had a longer progression-free survival than patients with high programmed cell death protein-1 ligand ($P =$ .038). In the gemcitabine and taxane groups, patients with low programmed cell death protein-1 ligand had a longer progression-free survival than patients with high programmed cell death protein-1 ligand ($P =$ .047). There was a significant correlation between neutrophil-to-lymphocyte ratio and programmed cell death protein-1 ligand levels. In the groups with high programmed cell death protein-1 ligand, patients with low neutrophil-to-lymphocyte ratio levels had higher overall survival than patients with high neutrophil-to-lymphocyte ratio level ($P =$ .043). Also, there was a significant difference between the overall survival patients with low and high platelet-to-lymphocyte ratio levels ($P =$ .520).

CONCLUSION: In patients with metastatic non-small cell lung cancer whose programmed cell death protein-1 ligand levels and neutrophil-to-lymphocyte ratio levels are low, immunogenic chemotherapies such as gemcitabine and taxane can be tried as an alternative treatment.

KEYWORDS: Chemotherapy, lung cancer, NLR, PDL-1, PFS, PLR
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INTRODUCTION

Lung cancer is the most common cause of cancer-related deaths worldwide.[1] Approximately 85% of lung cancers are non-small cell lung cancer (NSCLC), and 80% of patients are diagnosed as advanced stage.[2-3] Conventional cytotoxic chemotherapies have been used as the standard treatment in lung cancer until recently.[4] Although the importance of targeted agents and immunotherapy has started to increase, chemotherapies are still the main therapy for most patients in the treatment of lung cancer.[4] While chemotherapies were thought to only cause immunosuppression in the past, it is now accepted that chemotherapies can increase tumor immunity.

Treatments with immune checkpoint inhibitors which are developed against PD-1 (programmed cell death protein-1) and programmed cell death protein-1 ligand (PDL-1) that is a ligand of this protein led to improvements in response rates and survival times in patients.[5] In most of the immunotherapy studies in the literature, it has been shown that there is a relationship between tissue PDL-1 expression level and clinical efficacy. Despite these studies showing the relationship between PDL-1 expression levels and response to treatment, PDL-1 still remains a controversial biomarker of immunotherapy. The differences in the tests applied to determine the level of PDL-1, the different cut-off values for expressions among different immunotherapy agents, and the heterogeneity of PDL-1 expression in the tumor may be the reasons for discussion of PDL-1 as a biomarker.[6]

In the literature, there are studies showing that PDL-1 expression, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) are also associated with poor survival in many tumor types.[7-9] In these studies, higher NLR and PLR values for NSCLC were associated with worse survival.[10,11] However, the effects of NLR and PLR values on survival in patients with different PDL-1 levels have not been clearly demonstrated.

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The aim of this study is to show the effect of PDL-1 level on PFS provided by chemotherapy agents in first-line treatment of patients with metastatic NSCLC and to determine whether there is a relationship between PDL-1 level, NLR, and PLR.

MATERIAL AND METHODS

Patients

In this study, the data of 158 patients who received chemotherapy with a diagnosis of metastatic NSCLC between 2016 and 2021 at our hospital were evaluated retrospectively. Tumor histology, metastasis sites, treatments received, pre-treatment hematological parameters, and follow-up periods of the patients were recorded. Programmed cell death protein-1 ligand expression levels of the patients evaluated by immunohistochemistry (IHC) method were recorded. None of the patients included in the study received immunotherapy.

DACO 22C3 PDL-1 antibody was used for IHC analysis in tumor tissues and the presence of 1% membranous immuno-histochemical staining in tumor cells was determined as the cut-off value for PDL-1 expression level. Immunohistochemistry was applied using PD-L1 kit (PD-L1 IHC 22C3 PharmDX; Dako, Carpinteria, CA, USA) according to the manufacturer's instructions. This antibody was preferred since the Food and Drug Administration approved this antibody as a companion test to determine the applicability of pembrolizumab therapy. Sections of 4 μm from each block were cut and stained with 22C3 stain on the Dako Link-48 automated staining system. For PDL-1 positivity, a threshold value of 50% was considered. Patients below and above this level were classified as low and high level for PDL-1.

In PDL-1 expression groups, therapy response and survival times were compared according to chemotherapy type and tumor histological type. In addition, the relationship between PDL-1, NLR, PLR, and the effect of NLR and PLR values on overall survival were evaluated in PDL-1 expression groups.

Statistical Analysis

The Statistical Package for Social Sciences version 22.0 software (IBM Corp.; Armonk, NY, USA) was used for statistical analysis. Whether the data were normally distributed was determined by Kolmogorov–Smirnov and Shapiro–Wilk tests. Student's t-test and Mann-Whitney U test were used to compare continuous variables between groups, and Chi-square test and Fisher's exact test were used to compare categorical variables. Receiver operating characteristic (ROC) analysis and area under the curve (AUC) values were used to determine the roles of NLR and PLR parameters in predicting PFS and OS. Kaplan-Meier test was used for survival analysis. Results are presented as mean ± standard deviation, median (min-max), and number (percentage). A P value of <.05 was considered statistically significant in all statistical analyses.

## RESULTS

Patient Characteristics

The mean age of the patients was 63.8 ± 9.3 years (41-81). The demographic and clinical characteristics of the patients are shown in Table 1. Histological subtypes of the patients

Table 1. Demographic and Clinical Characteristics of the Patients

Age (years) mean ± SD (min-max)	63.8 ± 9.3 (41.0-81.0)
Gender, n (%)	
Female	37 (23.4%)
Male	121 (76.6%)
Smoking, n (%)	
Yes	90 (56.9%)
No	68 (43.0%)
Histological type, n (%)	
Adeno Ca	113 (71.5%)
Squamoz Ca	36 (22.8%)
Other	9 (5.7%)
Metastasis zone, n (%)	
Liver	13 (8.2%)
Lungs	31 (19.6%)
Bone	35 (22.2%)
Lymph	88 (55.7%)
Brain	16 (10.1%)
Surrenal	15 (9.5%)
Other	34 (21.5%)
PDL-1 groups, n (%)	
<50	73 (46.2%)
≥50	85 (53.8%)
Treatment groups, n (%)	
Taxane	61 (38.6%)
Pemetrexet	17 (10.8%)
Vinorelbine	24 (15.2%)
Gemcitabine	23 (14.6%)
Other	33 (20.9%)

PDL-1, programmed cell death protein-1 ligand; SD, standard deviation.

were 71.5% adenocarcinoma and 22.8% squamous cell cancer. In our study, 46.2% of patients had low PDL-1 levels and 53.8% of patients had high PDL-1 levels. Programmed cell death protein-1 ligand (PDL-1) levels were high in 52% and low in 48% of patients with adenocarcinoma histology. These rates were 43.7% and 56.5% in patients with squamous cell carcinoma histology, respectively. The most common metastasis site was lymph node (55.7%), followed by bone (22.2%), lung (19.6%), brain (10.1%), and liver (8.2%). In addition to the first-stage platinum-based chemotherapy treatment, taxane was added to 38.6% of patients, vinorelbine to 15.2%, gemcitabine to 14.6%, and pemetrexed to 10.8%. After first-line treatment, 84 patients (53.2%) progressed and 50% of these patients received treatment in the next steps. In the second-line treatment, 24 patients received pemetrexet (10 patients low PDL-1, 14 patients high PDL-1), 22 patients taxane (11 patients low PDL-1, 11 patients high PDL-1), 9 patients gemcitabine (5 patients low PDL-1, 4 patients high PDL-1), and 7 patients vinorelbine (3 patients low PDL-1, 4 patients high PDL-1).
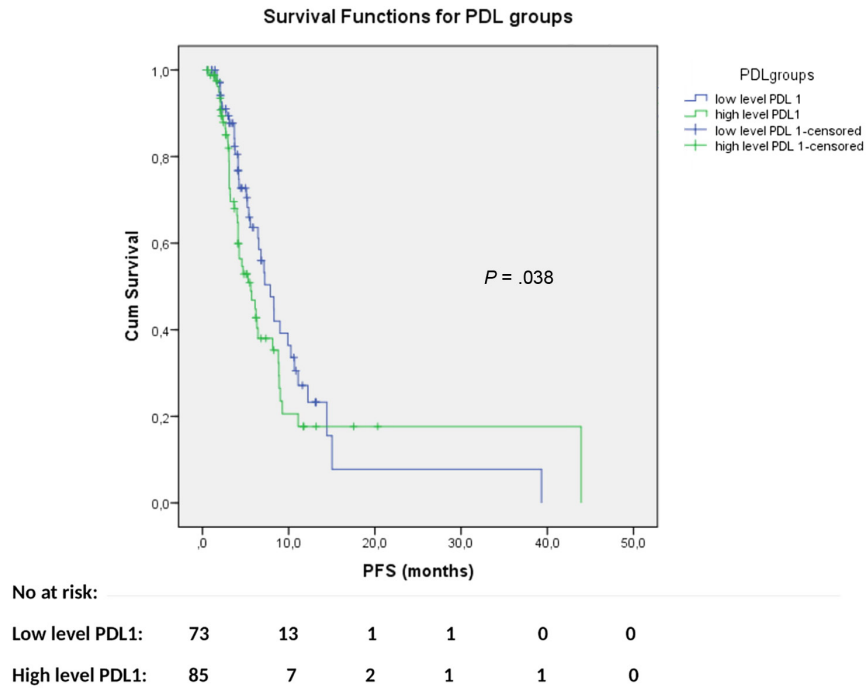
Figure 1. Comparison of patients' PFS according to PDL-1 expression groups by Kaplan-Meier analysis. PFS, progression-free survival; PDL-1, programmed cell death protein-1 ligand.

Progression Free Survival and Overall Survival

In all patients, PFS was 5.6 months (95% CI, 0.6-43.9) and OS was 18.8 months (95% CI, 0.7-155.5). When PFS and OS were evaluated according to PDL-1 expression level, patients with low PDL-1 expression had a statistically significant longer PFS than patients with high PDL-1 expression (7.9 months (95% CI, 5.9-9.8) and 5.6 months (95% CI, 3.6-7.5), respectively, $P = .038$) (Figure 1). There was no statistically significant difference in the evaluation of OS between the groups (26.4 months vs 26.3 months, respectively, $P = .750$). When OS was compared according to gender, histology type, and metastasis type, no statistically significant difference was found (Table 2). In all histological types, although the duration of PFS was higher in patients with low PDL-1 levels, no statistically significant difference was found (Table 3).

In the gemcitabine and taxane groups, PFS of the patients with low PDL-1 expression were found to be statistically significantly longer than the patients with high PDL-1 expression ($P = .047$ and $P = .048$, respectively) (Figure 2). The PFSs of all used agents according to the PDL-1 expression level are presented in Table 4.

Relationship Between Hematological Parameters and Programmed Cell Death Protein-1 Ligand

While there was a statistically significant but low correlation between NLR and PDL-1 level (r = 0.184 and $P = .021$), there was no correlation between PLR and PDL-1 level (r = -0.062 and $P = .443$). When the correlation between NLR and PLR level and OS and PFS was evaluated, a statistically significant negative correlation was observed only between NLR level and OS and PFS (r = -0.184 and $P = .021$, r = −0.161 and $P = .045$, respectively).

In patients with high PDL-1 expression, there was a statistically significant but weak correlation between NLR level

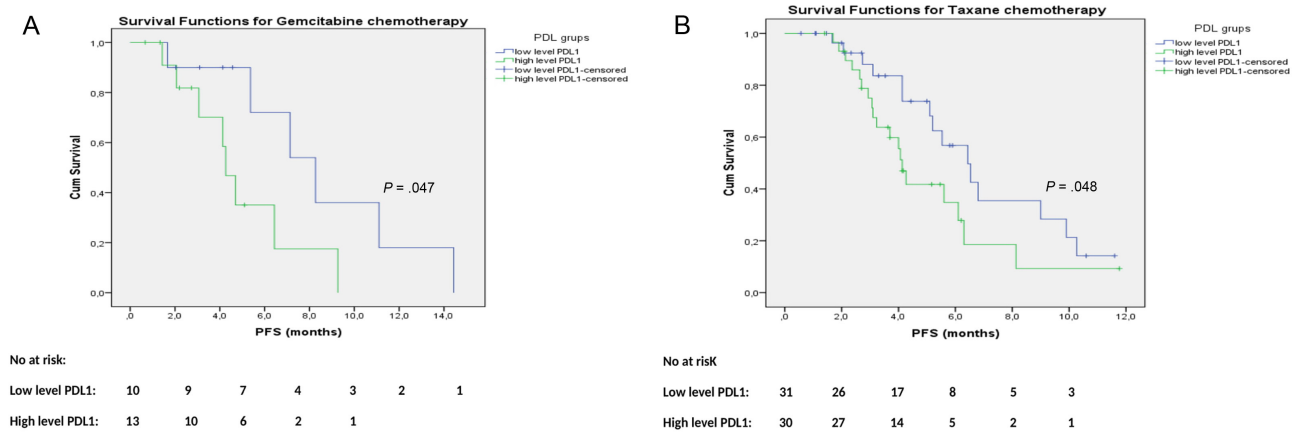**Table 2.** Effects of Clinical and Demographic Characteristics of Patients on OS by Kaplan–Meier Analysis

	OS (Months)	95% CI	P
Gender, n (%)			.671
Female	18.7 ± 2.7	13.6-23.8	
Male	17.9 ± 2.6	12.5-23.8	
Histological type, n (%)			.515
Adeno Ca	16.7 ± 2.0	12.9-20.5	
Squamoz Ca	21.7 ± 4.2	13.4-30.0	
Metastasis			.899
Solitary	16.7 ± 2.8	11.2-22.2	
Multiple	19.5 ± 3.0	13.6-25.4	

OS, overall survival.

Table 3. Effect of PDL-1 Level on PFS According to Tumor Histological Type by Kaplan-Meier Analysis

Tumor Histological Type	PDL Groups	Estimated Time PFS (Months)	95% CI	P
Adeno Ca	Low level PDL-1	7.2 ± 0.9	5.6-8.9	.143
	High level PDL-1	5.6 ± 1.0	3.5-7.7	
Squamous carcinoma	Low level PDL-1	8.3 ± 2.0	4.4-12.1	.197
	High level PDL-1	4.7 ± 0.9	2.9-6.5	

PFS, progression free survival; PDL-1, programmed cell death protein-1 ligand.

47

A

Survival Functions for Gemcitabine chemotherapy

B

Survival Functions for Taxane chemotherapy

Figure 2. (a, b) Effect of PDL-1 expression levels on PFS in patients receiving Gemcitabine and Taxane chemotherapy by Kaplan–Meier analysis. PFS, progression-free survival; PDL-1, programmed cell death protein-1 ligand.

and OS (r = -0.220, *P* = .043), but there was no correlation between NLR level and PFS (r = -0.159, *P* = .150). Also, there was no correlation between the PLR level and PSF and OS (r = -0.115, *P* = .294 and r = 0.071, *P* = .520, respectively).

Evaluation of NLR and PLR in predicting mortality in low and high PDL-1 expression groups are presented in Table 5 (Figure 3).

When the effect of high and low NLR and PLR on OS in patients with high and low PDL-1 expression was evaluated, in the group with high PDL-1 expression, patients with low NLR levels had higher OS than patients with high NLR levels (47.2 months (95% CI, 22.5-71.8) and 14.8 months (95% CI, 11.0-18.5), *P* = .035, respectively) (Figure 4). In addition, there was a statistically significant difference between the OS of patients with low and high PLR levels (97.5 months (95% CI, 70.5-124.5) vs. 21.7 months (95% CI, 16.1-27.3) respectively, and *P* = .033).

DISCUSSION

Programmed cell death protein-1 ligand expression rate in patients with NSCLC has been shown to be between 30% and 60%. Many meta-analyzes have shown that patients with high PDL-1 expression have lower survival rates.[12-14] However, in the literature, there are studies by Velcheti et al[15] showing that high PDL-1 expression leads to better survival (60.0 months vs 27.8 months, *P* = .037) in both Greek and Yale cohorts. However, the effect of PDL-1 expression on survival could not be demonstrated in the 320 and 170 case series of Schmidt and Tang.[16,17] Similarly, in our study, a significant effect of PDL-1 expression on OS could not be demonstrated. When the subgroups were examined, no OS difference was observed in terms of tumor histology, gender, metastasis location, and number of metastases. However, unlike these series, in our study, it was observed that the PFS duration was longer in the low level PDL-1 group compared to the high level PDL-1 group. The reason why PFS is better and this is not reflected in OS may be that the majority of our patients could not receive treatment after progression.

In recent years, many markers have been investigated to predict the effectiveness of chemotherapies in lung cancer patients.[18] Although there are studies indicating that PDL-1 is a biomarker showing immunotherapy response, its predictive value for chemotherapy is unknown. Our study showed that low PDL-1 expression provides better

Table 4. The Effect of PDL-1 Level on PFS According to the Type of Chemotherapy Used

Chemotherapy	PDL Groups	Estimated Time PFS (Months)	95% CI	*P*
Taxane	Low level PDL-1	6.4 ± 0.9	4.8-8.1	**.048**
	High level PDL-1	4.1 ± 0.2	3.8-4.5	
Pemetrexed	Low level PDL-1	7.2 ± 3.9	0.0-14.9	.674
	High level PDL-1	5.7 ± 1.7	2.4-9.0	
Vinorelbin	Low level PDL-1	9.6 ± 1.4	6.9-12.4	.143
	High level PDL-1	5.5 ± 1.1	3.4-7.5	
Gemcitabine	Low level PDL-1	8.3 ± 1.6	5.1-11.4	**.047**
	High level PDL-1	4.3 ± 0.4	3.5-5.1	
Others	Low level PDL-1	15.0 ± 3.5	8.2-21.9	.605
	High level PDL-1	43.9 ± 0.0	-	

Bold values are statistically significance.

PFS, especially in gemcitabin and taxane-based regimens. However, no relationship was shown between PDL-1 level and PFS in pemetrexet treatment. It is known that cytotoxic chemotherapies are effective in regulating the immune microenvironment. It has been clearly demonstrated in a study that immunogenic cell death and T cell infiltration occur with the application of neoadjuvant chemotherapy in early stage breast cancer.[19] However, which agent, by which mechanism, and in what way intensity plays a role in immune microenvironment modulation have not yet been clarified. There are studies showing that gemcitabine and taxanes increase PDL-1 levels by increasing the levels of activating immune receptor expressed by NK and effector T cells (NKG2D) ligands and stimulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB) and signal transducer and activator of transcription 3 (STAT3) signaling pathways.[20-22] The use of gemcitabine in combination with immunotherapy produced a synergistic clinical response in the preclinical mesothelioma model resistant to gemcitabine and immunotherapy monotherapy and broke immunotherapy resistance.[23] It has been observed that pemetrexet has an immunogenic effect by inducing more immunogenic cell death.[24]

As the immune responses associated with the tumor are evaluated, it has come to the fore whether different inflammation markers can be used as a prognostic marker in NSCLC. Neutrophil-to-lymphocyte ratio has been shown to be an early marker of inflammation in NSCLC and in many different solid tumors. Moreover, if an increase in NLR is observed under treatment, it is thought that it may indicate treatment failure.[25,26] It has been found that high NLR increases the angiogenesis of the tumor environment (via VEGF and IL-18), inhibits cell apoptosis, and thus increases tumorigenesis and leads to worse results.[27,28] Liu et al[29] retrospectively evaluated 325 patients with advanced stage lung cancer in their study and showed that PFS and OS were longer in the low NLR group compared to the high NLR group.[29] Similarly, in our study, both PFS and OS were found to be worse in patients with higher NLR. Moreover, in our study, there was a relationship between the NLR value and OS, especially in patients with high PDL-1 expression. While there are studies in the literature showing that both PDL-1 and NLR levels are related to prognosis separately, this is the first study showing the relationship of PDL-1 expression with NLR level and evaluating the effect of NLR on survival times in patients grouped according to PDL-1 expression levels.
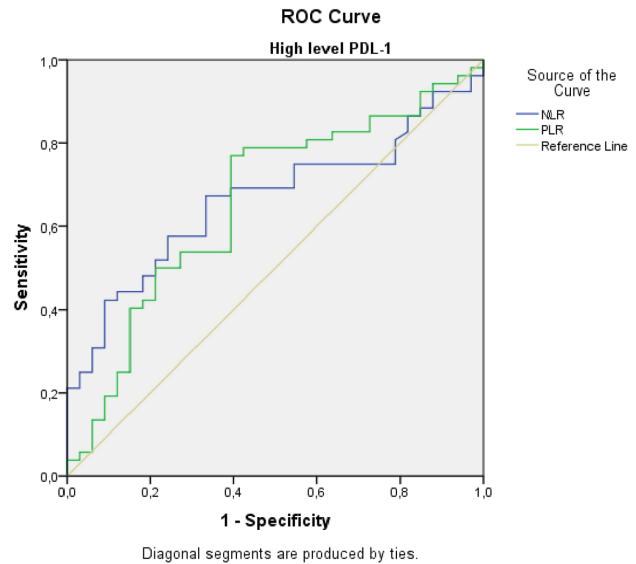
Figure 3. Prediction of mortality in patients with high PDL-1 levels for NLR and PLR by ROC analysis. PDL-1, programmed cell death protein-1 ligand; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Since platelets increase cell proliferation and have pro-tumor activity by stimulating growth factors, an increase in PLR value negatively affects survival.[29,30] Similar to the literature, patients with low PLR values were found to have longer OS durations than patients with high PLR values in our study. However, no relationship was observed between PDL-1 level and PLR.

Currently, pembrolizumab and nivolumab targeting PD-1 and atezolizumab, avelumab, and durvalumab targeting PDL-1 are among the immunotherapy options. In metastatic NSCLC, current immunotherapy options can be used in combination with chemotherapy or as a single agent. While immunotherapy monotherapy was appropriate in patients with PDL-1 ≥50, combinations of immunotherapy+chemotherapy showed superior survival in patients with PDL-1 <50.[31,32] Immunotherapy may cause some immune-related side effects that require a variety of therapeutic approaches. The most common side effects are rash and diarrhea. However, it can be seen in serious immune-related side effects such as hypophysitis, pneumonia, thyroiditis, and hepatitis.[33] The most important problem in immunotherapy, as in targeted agents, is the development of resistance caused by the activation of the epidermal growth factor receptor

	AUC	95% CI	P	Cut-Off	Sensitivity	Specificity
Table 5. Evaluation of NLR and PLR Predicting Mortality in PDL-1 Expression Groups by ROC Analysis						
Low level PDL-1						
NLR	0.595	0.466-0.725	.198	-	-	-
PLR	0.438	0.302-0.573	.400	-	-	-
High level PDL-1						
NLR	0.673	0.559-0.788	.008	3.22	70.6%	60.6%
PLR	0.658	0.537-0.779	.015	142.02	78.4%	57.6%

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; AUC, area under the curve; PDL-1, programmed cell death protein-1 ligand.
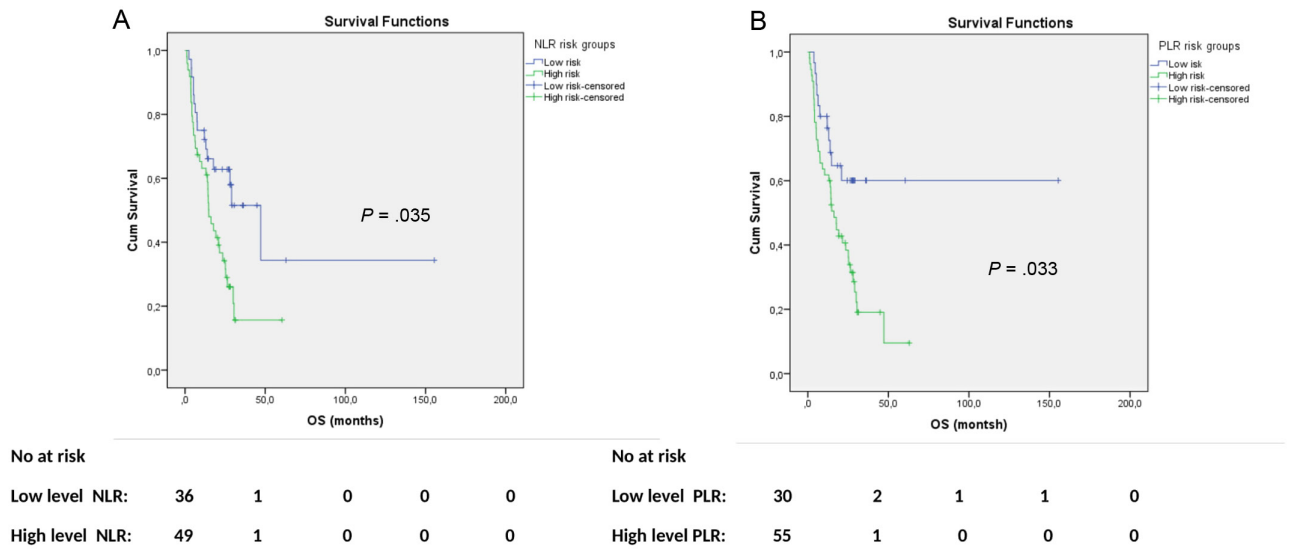
Figure 4. (a, b) The effect of NLR and PLR on OS in the group with high PDL-1 level by Kaplan–Meier analysis. PDL-1, programmed cell death protein-1 ligand; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; OS, overall survival.

(EGFR) pathway. Especially since EGFR pathway activation is seen more frequently in Asians than in western societies, resistance may develop more frequently in these patient groups. However, we do not have sufficient data in terms of immunotherapy responses and genetic disposition. Despite the indisputable efficacy of immunotherapy in metastatic NSCLC, these treatments cannot be used in all patients as the cost of treatment is a major concern in many countries.[34] Our study demonstrated the efficacy of chemotherapy in patients with advanced NSCLC whose expression of PDL-1 in tumor cells was evaluated.

Our study has some limitations. The most important of these limitations are that it is designed retrospectively and the number of patients in the chemotherapy arms is not equal. As another limitation, immunotherapy, which is the standard treatment, could not be given to these patients due to the health policies of our country, and the patients received chemotherapy. Since immunotherapy was not used in any patient, only the prognosis effects of PDL-1, NLR, and PLR were evaluated in patients receiving chemotherapy in our study.

CONCLUSION

In conclusion, according to the findings of this study, taxane and gemcitabine-based therapies provided a better PFS compared to pemetrexet-based treatments in patients with low PDL-1 expression. In addition, it was found that patients with low NLR levels had longer survival times and there was a relationship between NLR and PDL-1. Based on these data, cytotoxic chemotherapy options can be tried as an alternative treatment due to positive effects on survival times, especially in patients whose PDL-1 expression level and NLR level are low and who cannot reach immunotherapy. Immunogenic chemotherapies such as gemcitabine and taxane, which play a role in immune environment modulation, may be candidates for this treatment.

REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65(2):87-108. [CrossRef]
2. Herbst RS, Heymach JV, Lippman SM. Lung cancer. *N Engl J Med.* 2008;359(13):1367-1380. [CrossRef]
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016;66(1):7-30. [CrossRef]
4. Hellmann MD, Li BT, Chaft JE, Kris MG. Chemotherapy remains an essential element of personalized care for persons with lung cancers. *Ann Oncol.* 2016;27(10):1829-1835. [CrossRef]
5. Liu X, Guo CY, Tou FF, et al. Association of PD-L1 expression status with the efficacy of PD-1/PD-L1 inhibitors and overall survival in solid tumours: a systematic review and meta-analysis. *Int J Cancer.* 2020;147(1):116-127. [CrossRef]
6. Zhang Y, Wang L, Li Y, et al. Protein expression of programmed death 1 ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma. *Onco Targets Ther.* 2014;7:567-573. [CrossRef]
7. Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. *Crit Rev Oncol Hematol.* 2013;88(1):218-230. [CrossRef]
8. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic

review and meta-analysis. *J Natl Cancer Inst.* 2014;106(6):dju124. **[CrossRef]**

9. Templeton AJ, Ace O, McNamara MG, et al. Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2014;23(7):1204-1212. **[CrossRef]**

10. Zhang H, Xia H, Zhang L, Zhang B, Yue D, Wang C. Clinical significance of preoperative neutrophil-lymphocyte vs platelet-lymphocyte ratio in primary operable patients with non-small cell lung cancer. *Am J Surg.* 2015;210(3):526-535. **[CrossRef]**

11. Proctor MJ, McMillan DC, Morrison DS, Fletcher CD, Horgan PG, Clarke SJ. A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer. *Br J Cancer.* 2012;107(4):695-699. **[CrossRef]**

12. Pan ZK, Ye F, Wu X, An HX, Wu JX. Clinicopathological and prognostic significance of programmed cell death ligand1 (PD-L1) expression in patients with non-small cell lung cancer: a meta-analysis. *J Thorac Dis.* 2015;7(3):462-470. **[CrossRef]**

13. Wang A, Wang HY, Liu Y, et al. The prognostic value of PD-L1 expression for non-small cell lung cancer patients: a meta-analysis. *Eur J Surg Oncol.* 2015;41(4):450-456. **[CrossRef]**

14. Zhong A, Xing Y, Pan X, Shi M, Xu H. Prognostic value of programmed cell death-ligand 1 expression in patients with non-small-cell lung cancer: evidence from an updated meta-analysis. *Onco Targets Ther.* 2015;8:3595-3601. **[CrossRef]**

15. Velcheti V, Schalper KA, Carvajal DE, et al. Programmed death ligand-1 expression in non-small cell lung cancer. *Lab Invest.* 2014;94(1):107-116. **[CrossRef]**

16. Schmidt LH, Kümmel A, Görlich D, et al. PD-1 and PD-L1 expression in NSCLC indicate a favorable prognosis in defined subgroups. *PLoS One.* 2015;10(8):e0136023. **[CrossRef]**

17. Tang Y, Fang W, Zhang Y, et al. The association between PD-L1 and EGFR status and the prognostic value of PD-L1 in advanced non-small cell lung cancer patients treated with EGFR-TKIs. *Oncotarget.* 2015;6(16):14209-14219. **[CrossRef]**

18. Coate LE, John T, Tsao MS, Shepherd FA. Molecular predictive and prognostic markers in non-small-cell lung cancer. *Lancet Oncol.* 2009;10(10):1001-1010. **[CrossRef]**

19. Aoto K, Mimura K, Okayama H, et al. Immunogenic tumor cell death induced by chemotherapy in patients with breast cancer and esophageal squamous cell carcinoma. *Oncol Rep.* 2018;39(1):151-159. **[CrossRef]**

20. Chang LS, Yan WL, Chang YW, et al. Gemcitabine enhances antitumor efficacy of recombinant lipoimmunogen-based immunotherapy. *Oncoimmunology.* 2016;5(3):e1095433. **[CrossRef]**

21. Doi T, Ishikawa T, Okayama T, et al. The JAK/STAT pathway is involved in the upregulation of PD-L1 expression in pancreatic cancer cell lines. *Oncol Rep.* 2017;37(3):1545-1554. **[CrossRef]**

22. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med.* 2015;373(2):123-135. **[CrossRef]**

23. Lara PT, Cecconi, Hiltbrunner S, et al. Gemcitabine synergizes with immune checkpoint inhibitors and overcomes resistance in a preclinical model and mesothelioma patients. *Clin Cancer Res.* 2018;24(24):6345-6354. **[CrossRef]**

24. Zhang P, Bao Z, Xu L, et al. PD-L1 expression indicates favorable prognosis for advanced lung adenocarcinoma patients treated with pemetrexed. *Oncotarget.* 2017;8(39):66293-66304. **[CrossRef]**

25. Tomıta M, Shımızu T, Ayabe T, Yoneı A, Onıtsuka T. Preoperative neutrophil to lymphocyte ratio as a prognostic predictor after curative resection for non-small cell lung cancer. *Anticanc Res.* 2011;31(9):2995-2998.

26. Yıldırım F, Sevimli N, Türk M, Demirci NY, Yurdakul AS, Oztürk C. Does neutrophil-to-lymphocyte ratio have a role among the other prognostic factors of nonsmall cell lung cancer? *Eurasian J Pulmonol.* 2019;21(3):187-192. **[CrossRef]**

27. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature.* 2011;480(7378):480-489. **[CrossRef]**

28. Lee KH, Lee CH, Jeong J, Jang AH, Yoo CG. Neutrophil elastase differentially regulates interleukin 8 (IL-8) and vascular endothelial growth factor (VEGF) production by cigarette smoke extract. *J Biol Chem.* 2015;290(47):28438-28445. **[CrossRef]**

29. Liu ZL, Zeng TT, Zhou XJ, et al. Neutrophil-lymphocyte ratio as a prognostic marker for chemotherapy in advanced lung cancer. *Int J Biol Markers.* 2016;31(4):e395-e401. **[CrossRef]**

30. Xu F, Xu P, Cui W, et al. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios may aid in identifying patients with non-small-cell lung cancer and predicting tumor-node-metastasis stages. *Oncol Lett.* 2018;16(1):483-490. **[CrossRef]**

31. Zhou GW, Xiong Y, Chen S, Xia F, Li Q, Hu J. Anti-PD-1/PD-L1 antibody therapy for pretreated advanced nonsmall cell lung cancer: a meta-analysis of randomized clinical trials. *Medicine.* 2016;95(35):e4611. **[CrossRef]**

32. Tanvetyanon T, Creelan BC, Antonia SJ. The safety and efficacy of nivolumab in advanced (metastatic) non-small cell lung cancer. *Expert Rev Anticancer Ther.* 2016;16(9):903-910. **[CrossRef]**

33. Foller S, Oppel-Heuchel H, Fetter I, Winkler Y, Grimm MO. Adverse events of immune checkpoint inhibitors [in German]. *Urologe A.* 2017;56(4):486-491. **[CrossRef]**

34. Fathi Z, Syn NL, Zhou JG, Roudi R. Molecular epidemiology of lung cancer in Iran: implications for drug development and cancer prevention .*J Hum Genet.* 2018;63( ):783-794. **[CrossRef]**