Original Article

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Neutrophil to lymphocyte ratio as a potential predictive marker for treatment with pembrolizumab as a second line treatment in patients with non-small cell lung cancer

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SUMMARY The aim of this multicentric retrospective study is to evaluate the predictive and prognostic performance of neutrophil to lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and their dynamics in patients with non-small cell lung cancer (NSCLC) treated with pembrolizumab as a second line. Patients with metastatic NSCLC (n = 119), whose tumors expressed programmed death-ligand 1 (PD-L1) ≥ 1%, were retrospectively analyzed between Apr 2017 and Apr 2019. All patients received platinum-containing chemotherapy as a first line treatment. Pre-treatment NLR was calculated by dividing the number of neutrophils by the number of lymphocytes in peripheral blood before the first pembrolizumab infusion. Progression free survival (PFS) and overall survival (OS) was compared by Kaplan-Meier method and Cox Proportional Hazard model. Patients with NLR > 5 before immunotherapy showed significantly shorter mean PFS of 6.86 months (95% CI: 5.81-7.90) as compared to those with NLR ≤ 5 of 18.82 months (95% CI: 15.87-21.78) (long rank test p < 0.001). Furthermore in the multivariate analysis, only NLR > 5 was an independent predictive factor for shorter PFS (HR: 4.47, 95% CI: 2.20-9.07, p < 0.001). In multivariate analysis, presence of bone metastases (HR: 2.08, 95% CI: 1.10-4.94, p = 0.030), NLR > 5 before chemotherapy (HR: 8.09, 95% CI: 2.35-27.81, p = 0.001) and high PLR before chemotherapy (HR: 2.81, 95% CI: 1.13-6.97, p = 0.025) were found to be independent negative prognostic factors for poor OS. Our data suggests that NLR ≤ 5 is a potential predictive marker, which may identify patients appropriate for immunotherapy as a second line treatment.

Keywords neutrophil to lymphocyte ratio, predictive marker, immunotherapy

1. Introduction

Lung cancer is the leading cause of cancer death worldwide (*1*). Non-small cell lung cancer (NSCLC) accounts for about 80% of lung cancers. Recently, immunotherapy has turned out to be of great interest to researchers, especially with its promise to treat various forms of cancer including NSCLC (*2*). Human immune checkpoint-inhibitor antibodies inhibit the program death (PD-1) receptor or its ligand PD-L1. This helps to improve antitumor immunity. However, for different reasons, platinum-based chemotherapy still remains the first-line treatment or at least part of it for the majority of patients without targetable oncogenic driver alterations (*3,4*). In the phase II/III KEYNOTE-010 study, pembrolizumab significantly prolonged overall survival over docetaxel as second line therapy in advanced NSCLC (*5*). Despite these advances in treatment and the increased knowledge of the molecular pathways, our understanding why some people benefit from treatment with immunotherapy while others do not, is not well clarified (*6,7*). Durable responses can be observed in these populations, although the percentage was often found to be lower than 20% (*8,9*). This is why it is

important to identify a biomarker, which will predict the response to checkpoint blockades so as to optimize patient clinical benefit.

Overexpression of PD-L1 is an important and widely-explored predictive biomarker for the response to PD-1/PD-L1 antibodies (*10*). Immune cells which are most commonly associated with tumor progression and poor prognosis include neutrophils, platelets, macrophages and regulatory T cells (*11,12*). Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) which are easily and usually performed in clinical practice, prove to be established strong prognostic markers associated with the worse OS in several tumor types including NSCLC in the pre-immunotherapy era (*13,14*). Limited studies suggested that high NLR and PLR predict poor response to nivolumab as a second line treatment (*15-17*).

The purpose of this retrospective study is to evaluate the predictive and prognostic performance of NLR, PLR and their dynamics in patients with NSCLC treated with pembrolizumab as a second line.

2. Materials and Methods

2.1. Patient selection

In this retrospective study we reviewed the cases of 119 patients from four centers in Bulgaria with metastatic NSCLC treated with pembrolizumab who have progressed after first line platinum-based chemotherapy between April 1, 2017 and April 30, 2019. The procedure was approved by the Scientific Research Ethics Committee at the Hospital "Nadezhda" in Sofia. Patients were eligible if they were ≥18 years old, and had histologically confirmed diagnosis of NSCLC in metastatic stage. Patients were Epidermal Growth Factor Receptor/Anaplastic lymphoma kinase (ALK) wildtype. All patients were in Eastern Cooperative Oncology Group -Performance status (ECOG-PS) < 2, and had disease progression after receiving one prior platinium-based systemic therapy for metastatic disease, with blood cell count and blood samples available. Patients were excluded if they had brain metastases (since the use of corticosteroids may compromise therapy), had autoimmune disease, symptomatic interstitial lung disease, systemic immunosuppression, or prior treatment with immune-stimulatory antitumor agents including checkpoint inhibitors. Patients did not show any clinical or computed tomography signs of active infection. Tumor PD-L1 status was required. Before starting a pembrolizumab treatment patients had at least 3 weeks free of treatment. Pembrolizumab was initially administered at 2 mg/kg intravenously (*i.v.*) over 60 minutes every 3 weeks and later at 200 mg *i.v.*, flat dose, every 3 weeks.

2.2. Data collection

Data collected included: demographics; smoking history; weight, height and body-mass index (BMI), histology; PD-L1 status; metastatic sites at initial diagnosis; description of first line treatment; date of progression (or last follow-up) as determined by radiology reports; and date of death or last follow-up. Peripheral blood samples were collected from patients included in the study the day of the first line chemotherapy administration at baseline and the day of first immunotherapy infusion upon progression. Hematological and biochemistry parameters of interest were absolute leukocyte (Leu), RDW (red blood cell distribution width), absolute neutrophil (ANC), absolute lymphocyte (ALC) and platelet (APC) counts, enabling calculation of NLR (Neutrophil to Lymphocyte Ratio – ANC/ALC) and PLR (Platelet to Lymphocyte ratio-APC/ALC). NLR1 and PLR1 were calculated before the first cycle of chemotherapy, NLR2 and PLR 2 – before the first pembrolizumab infusion. ΔNLR (NLR2-NLR1) and ΔPLR (PLR2-PLR1) were calculated. An NLR > 5 was considered elevated in accordance with earlier reports (*15,18,19*). The median value of NLR1 was 4.96. The median value of PLR was used to group cases into two categories of low (≤ median-200) and high (> median). Relative NLR and PLR change was analyzed: (calculated as % change ({[NLR2 / NLR1] -1} *100) and subsequently grouped in two groups (≥ 25% and < 25% increase).

The tumor PD-L1 protein expression level was examined in archived biopsy samples of the tumors using the PD-L1 immunohistochemistry (IHC) 28-8 pharmDx (Dako) kit (Agilent Technology). According to the manufacturer's kit criteria, cases with positive membranous staining of 1% or more of the tumor cells were defined as positive. In addition, we subdivided the positive group into expression categories - 50% or more, between 25-49% and between 1-24%.

2.3. Endpoints

The tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (ver.1.1) (RECIST 1.1) and clinical tumor response was assessed every 3 months. Patients were staged before treatment by performing computed tomography (CT). Clinical benefit rate (CBR) was defined as the proportion of patients with a partial response or stable disease for at least six months since no patients with complete response were recorded. Patients without clinical benefit (CB) were defined by a progression less than six months after treatment (chemotherapy or immunotherapy); patients with CB – the proportion of patients with radiological response or stabilization more than six months. Progression-free survival (PFS) was defined as the time elapsed between treatment initiation and tumor progression or death from any cause. Overall survival (OS) was defined as the interval between diagnosis

of the disease and death or date of last follow-up evaluation.

2.4. Statistical design and analysis

Data was managed and analyzed using SPSS software ver. 23. The demographic characteristics were expressed as frequencies and percentages for categorical variables and as medians and means with standard deviations for quantitative variables. The Mann–Whitney U test, χ^2 test and Spearman correlation were used to compare and evaluate the correlations between the biomarkers and the clinicopathological characteristics of the patients such as age, gender, PS (ECOG) - performance status (Eastern Cooperative Oncology Group). For interpretation of correlation test results, rho values were interpreted as follows, < 0.39, weak; 0.40-0.59, moderate; 0.60-0.79, strong; and ≥ 0.80, very strong. The Wilcoxon and McNemar tests were used to compare quantitative and categorical biomarkers values and their dynamics. The diagnostic accuracy of biomarkers was determined by obtaining the largest possible area under the curve (AUC) in receiver operating characteristic curve (ROC) analysis. AUC values: ≥ 0.9 are considered "excellent"; ≥ 0.80, "good"; ≥ 0.7, "fair"; and < 0.70, "poor". Survival curves according to the cutoff values of NLR and PLR were estimated using the Kaplan-Meier method, and differences were assessed using the log-rank test. Hazard ratios (HRs) and 95 percent confidence intervals for univariate and multivariate models were computed with the use of Cox proportional-hazards regression models. Two-tailed *p*-values (< 0.05) were considered significant.

3. Results

3.1. Baseline characteristics

This study includes 119 patients who after failure of first line chemotherapy have received anti PD-1-treatment with pembrolizumab. The clinical characteristics of the patients are summarized in Table 1. The mean age was 62.3 ± 7.9 years - most of the patients were men (62.2%), with nonsquamous histology (57.1%) and all patients exhibited ECOG PS 1. Lung was the most common metastatic site (78.2%), followed by pleural effusion (60.5%) and bone (40.3%). All patients were eligible for the examination of the tumor PD-L1 expression, of which 10 patients (8.4%) had more than 50% expression, 55 patients (46.2%) – between 25-49% expression and 54 patients (45.4%) – between 1-24%. Of all clinical-pathological characteristics of the patients only the presence of bone metastases was significantly related to CBR – Table 1.

3.2. Relation between CBR and immunological biomarkers

Blood biomarker results before first cycle of chemotherapy and before first pembrolizumab infusion are given at Table 2. Patients without CB in both chemotherapy and immunotherapy groups were characterized by significantly higher Leu, ANC, ALC and APC as compared to patients with CB. However, only NLR and PLR2 were related with CBR (Table 2).

The Wilcoxon test showed that ALC and APC did not change significantly from chemotherapy. Still, Leu

Table 1. Relations between baseline clinical-pathological characteristics of patients and Clinical Benefit (CB)

Characteristics		Immunotherapy			Chemotherapy		
	General Population (*n* = 119)	Patients without CB (*n* = 44)	Patients with CB (*n* = 75)	*p* value	Patients without CB (*n* = 30)	Patients with CB (*n* = 89)	*p* value
Age	62.3 ± 7.9	62.6 ± 7.3	62.0 ± 8.1	0.55	61.3 ± 7.1	62.7 ± 8.1	0.27
Gender				0.20			0.77
Men, *n* (%)	74 (62.2)	24	50		18 (60)	56 (62.9)	
Women, *n* (%)	45 (37.8)	20	25		12 (40)	33 (37.1)	
BMI	1.82 ± 0.14	1.74 ± 0.12	1.78 ± 0.15	0.21	1.86 ± 0.13	1.81 ± 0.14	0.068
ECOG PS							0.28
0	0	0	0		3	116	
1	119	74	45		73	46	
Histology				0.99			0.62
Non-squamous	68 (57.1)	25 (56.8)	43 (56.7)		16 (53.3)	52 (58.4)	
Squamous	51 (42.9)	19 (43.2)	32 (43.3)		14 (46.7)	37 (41.6)	
PD-L1 expression (%)				0.52			0.29
100-50	10 (8.4)	5 (11.4)	5 (6.8)		1 (3.3)	9 (10.1)	
49-25	55 (46.2)	18 (40.9)	37 (50)		17 (56.7)	38 (42.7)	
24-1	54 (45.4)	21(47.7)	33 (43.2)		12 (40)	42 (47.2)	
Metastatic sites							
Lung, *n* (%)	93 (78.2)	36 (81.8)	57 (75.7)	0.43	24 (80)	69 (77.5)	0.77
Pleura, *n* (%)	72 (60.5)	28 (63.6)	44 (58.1)	0.55	21 (70)	51 (57.3)	0.21
Liver, *n* (%)	29 (24.4)	12 (27.3)	17 (21.6)	0.48	7 (23.3)	22 (24.7)	0.87
Adrenal glands, *n* (%)	20 (16.8)	9 (20.5)	11 (13.5)	0.32	5 (16.7)	15 (16.9)	0.97
Bone, *n* (%)	48 (40.3)	24 (54.5)	24 (31.3)	0.012	18 (60)	30 (33.7)	0.011

Table 2. Patients' blood biomarker values before chemotherapy and immunotherapy and Clinical Benefit (CB)

Biomarker	Just before 1st CT				Just before 1st Pembrolizumab			
	General Population	Patients without CB (*n* = 30)	Patients with CB (*n* = 89)	*p* value	General Population	Patients without CB (*n* = 44)	Patients with CB (*n* = 75)	*p* value
Leu, mean ± SD	9.4 ± 2.6	10.2 ± 2.5	8.8 ± 2.6	0.002	10.1 ± 3.6	11.4 ± 3.6	9.4 ± 3.5	0.003
ANC, mean ± SD	6.5 ± 2.47	7.4 ± 2.2	5.8 ± 2.4	< 0.001	7.2 ± 3.4	8.7 ± 3.1	6.2 ± 3.3	< 0.001
ALC, mean ± SD	1.62 ± 0.64	1.41 ± 0.59	1.75 ± 0.64	0.001	1.64 ± 0.67	1.37 ± 0.56	1.82 ± 0.68	< 0.001
APC, mean ± SD	313.1 ± 85.1	311.3 ± 84.1	312 ± 88.2	0.84	323.1 ± 102.3	356.8 ± 103.6	304.1 ± 97.3	0.004
NLR, *n* (%)				< 0.001				< 0.001
≤ 5	61(50.8)	10 (22.7)	51 (68.5)		57 (47.9)	8 (18.2)	49 (66.2)	
> 5	58 (49.2)	34 (77.3)	24 (31.5)		62 (52.1)	36 (81.8)	26 (33.8)	
PLR				0.46				< 0.001
High		13 (43.3)	45 (51.1)			32 (72.7)	27 (36.5)	
Low		17 (56.7)	44 (48.9)			12 (27.3)	48 (63.5)	

Table 3. Receiver operating curve (ROC) analysis, using Neutrophil to Lymphocyte Ratio (NLR), Platelet-Lymphocyte Ratio (PLR) and their dynamics to differentiate patients between patients with and without clinical benefit. Diagnostic accuracy of biomarkers was determined by obtaining the largest possible area under the curve (AUC) in ROC analysis

Items	Biomarker	AUC 95% CI	*p* value	Sensitivity (%)	Specificity (%)
Chemotherapy Group	NLR1	0.651 (0.54-0.76)	0.014	64.8	63.3
	PLR1	0.575 (0.45-0.69)	0.21	61.4	56.7
Immunotherapy Group	NLR2	0.75 (0.66-0.85)	< 0.001	77.0	61.4
	PLR2	0.72 (0.63-0.82)	< 0.001	75.7	63.6
	ΔNLR	0.64 (0.53-0.75)	0.013	63.5	61.4
	ΔPLR	0.65 (0.54-0.75)	0.009	64.9	59.1

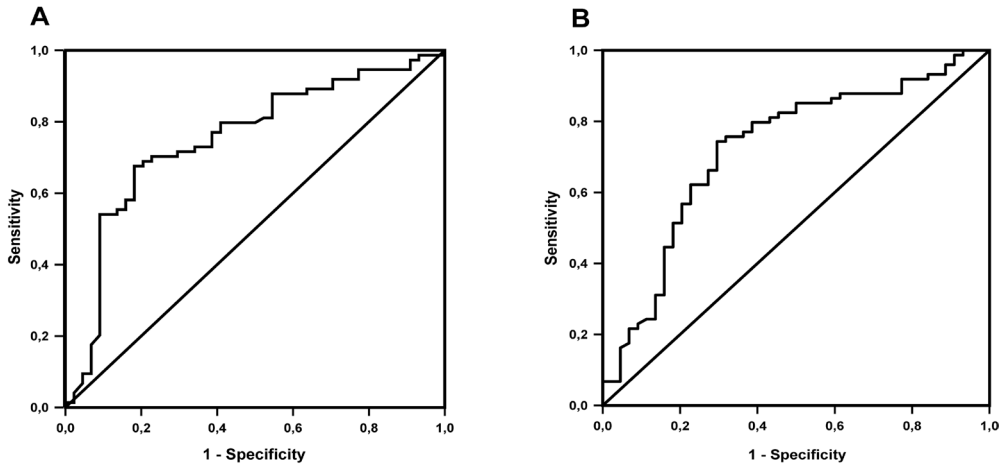

Figure 1. Receiver operating curve (ROC) analysis, using Neutrophil to Lymphocyte Ratio (NLR), Platelet-Lymphocyte Ratio (PLR) to differentiate between patients with and without clinical benefit. (A), NLR2; (B), PLR2.

and ANC significantly differ between the first cycle of chemotherapy and the first pembrolizumab infusion. The McNemar test showed that the proportion of patients with NLR > 5 and high PLR did not change significantly with chemotherapy treatment.

Significantly strong correlation was detected between NLR1 and PLR1 (rho = 0.737), NLR2 and PLR2 (rho = 0.774), and moderate correlation between Δ NLR and ΔPLR (rho = 0.494).

3.3. Immunological biomarkers and CBR

For the chemotherapy group CBR was 74.8%; for the immunotherapy group – 62.7%. ROC analysis was performed to explore the potential role of these biomarkers - NLR1, NLR2, PLR1, PLR2, ΔNLR, ΔPLR, as noninvasive ones for discrimination between patients with CB and without CB (Table 3). At the optimal cutoff values of the NLR1 and PLR1, only NLR1 could significantly, but poorly distinguish between patients with or without CB (AUC = 0.651, 95% CI: 0.54-0.76, p = 0.014) with a sensitivity of 64.8% and a specificity of 63.3%. In the immunotherapy group both biomarkers – NLR2 and PLR2, allowed significant but fair discrimination between patients with and without CB (Figure 1). NLR

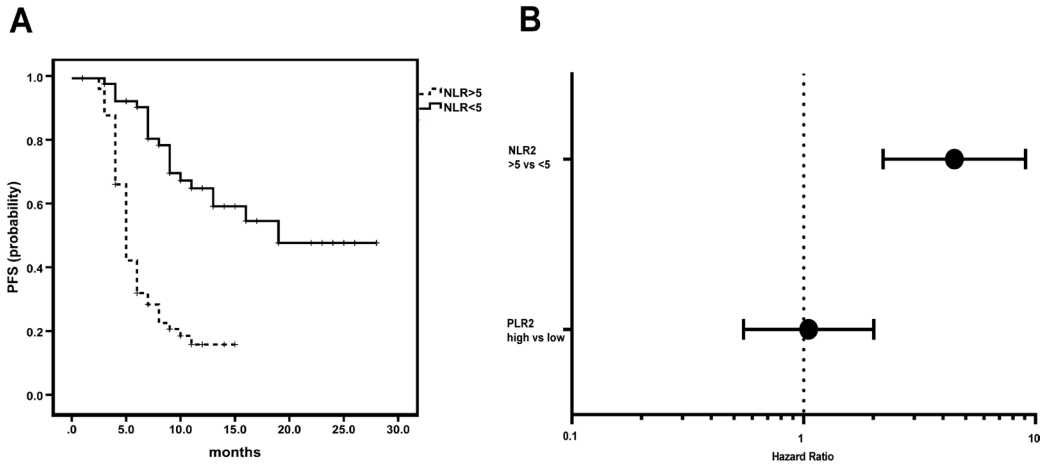

Figure 2. Kaplan-Maier estimates of progression free survival (PFS) in patients with NLR > 5 and NLR ≤ 5. (A), Patients with NLR > 5 showed significantly shorter mean PFS of 6.86 months (95% CI: 5.81-7.90) as compared to those with NLR ≤ 5 of 18.82 months (95% CI: 15.87-21.78) (long rank test $p < 0.001$); **(B)**, Forest plot showed progression-free survival across patients' subgroups. Hazard ratios are adjusted for gender, age, histology, PD-L1 expression, and ΔNLR and ΔPLR.

showed positive predictive value – 59% and negative predictive value – 86%. ΔNLR and ΔPLR could also discriminate between patients with and without CB, but poorly (Table 3).

Patients without CB had significantly higher values of ΔNLR (1.12 ± 2.2) and ΔPLR (49.6 ± 126.5) than patients with CB - ΔNLR (0.32 ± 1.95; $p = 0.013$) and ΔPLR (-7.7 ± 95.1; $p = 0.009$). The McNemar test showed that patients with CB differ between treatment with chemotherapy and immunotherapy ($p = 0.033$). Sixteen patients did not have clinical benefit either to chemotherapy, or to immunotherapy. They had significantly higher values of NLR (7.8 ± 2.08) and PLR (334.7 ± 91.9) than the rest of the patients (5.0 ± 3.4 and 221.4 ± 138.1). Twelve of them (75%) had bone metastasis.

3.4. Predictive and prognostic role of NLR and PLR in patients treated with pembrolizumab

Patients with NLR > 5 before immunotherapy showed significantly shorter mean PFS of 6.86 months (95% CI: 5.81-7.90) as compared to those with NLR ≤ 5 of 18.82 months (95% CI: 15.87-21.78) (long rank test $p < 0.001$) (Figure 2A). Patients with high PLR before immunotherapy showed also significantly shorter mean PFS of 11.01 months (95% CI: 8.46-13.57) as compared to those with low PLR of 15.96 months (95% CI: 13.08-18.84) (long rank test $p = 0.001$). Furthermore in the multivariate analysis, only NLR > 5 was an independent predictive factor for shorter PFS (HR: 4.47, 95% CI: 2.20-9.07, $p < 0.001$) (Table 4 and Figure 2B).

Patients with NLR > 5 had significantly shorter mean OS - 19.42 months (95% CI: 16.36-22.47) as compared to those with NLR ≤ 5 – 40.59 months (95% CI: 36.01-45.16) (log-rank test $p < 0.001$) (Figure 3A). Patients with high PLR had also shorter mean OS of 22.05 months (95% CI: 18.23-25.87) compared to patients with low PLR – 38.47 months (95% CI: 33.67-43.27) (long rank test $p < 0.001$) (Figure 3B). In univariate analysis squamous histology, presence of bone metastases, NLR > 5 and high PLR before chemo and immunotherapy, high RDW before chemotherapy, ΔNLR ≥ 25% and ΔPLR ≥ 25% were associated with worse OS. In multivariate analysis, however only presence of bone metastases (HR: 2.08, 95% CI: 1.10-4.94, $p = 0.030$), NLR > 5 before chemotherapy (HR: 8.09, 95% CI: 2.35-27.81, $p = 0.001$) and high PLR before chemotherapy (HR: 2.81, 95% CI: 1.13-6.97, $p = 0.025$) remained independent negative prognostic factors for poor OS (Table 5 and Figure 3D).

Table 4. Multivariate Cox regression analysis for predicting progression free survival

Marker	HR[a]-adjusted	95% CI	*p* value
NLR: > 5 *vs*. ≤ 5	4.47	2.20-9.07	< 0.001
PLR: High *vs*. Low	1.05	0.55-2.01	0.86

[a]Adjusted for gender, age, histology, PD-L1 expression, and ΔNLR and ΔPLR

4. Discussion

The current study found that patients with NLR > 5 had significantly shorter OS and PFS. Our study suggests that the proportion of patients with NLR > 5 and high PLR did not change significantly as a result of chemotherapy treatment and that NLR has an independent predictive value in NSCLC patients, treated with pembrolizumab as a second-line therapy. Patients with NLR > 5 had a lower chance to receive clinical benefit from immunotherapy. Furthermore, we demonstrated the negative prognostic role of high

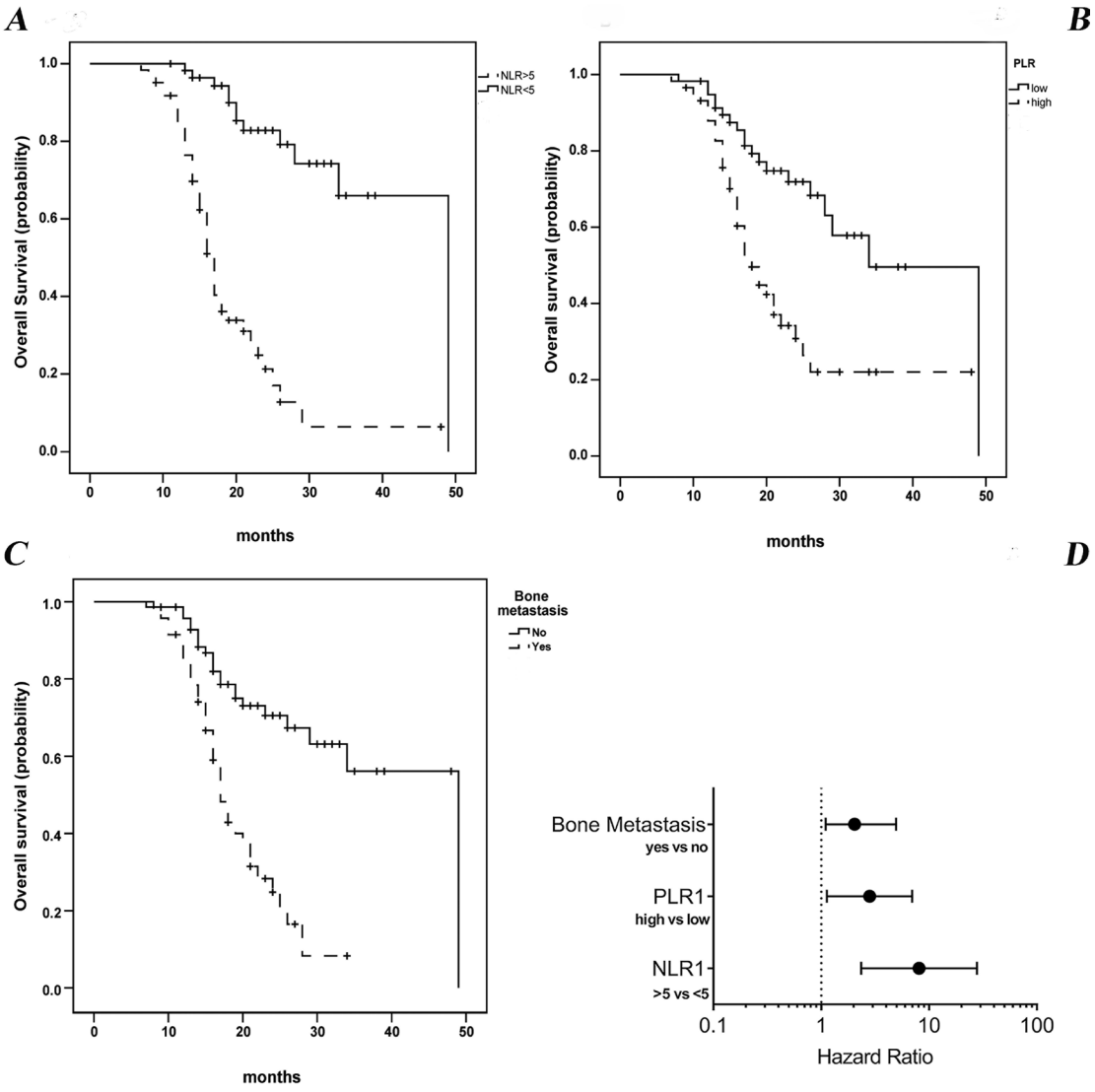

Figure 3. Kaplan-Maier estimates of overall survival (OS). (A), Patients with NLR > 5 had significantly shorter mean OS - 19.42 months (95% CI: 16.36-22.47) as compared to those with NLR ≤ 5 – 40.59 months (95% CI: 36.01-45.16) (log-rank test $p < 0.001$); **(B)**, Patients with high PLR had shorter mean OS of 22.05 months (95% CI: 18.23-25.87) compared to patients with low PLR – 38.47 months (95% CI: 33.67-43.27) (long rank test $p < 0.001$); **(C)**, Patients with bone metastasis had shorter mean OS of 19.24 months (95% CI: 17.01-21.45) compared to patients without bone metastasis – 36.60 months (95% CI: 32.12-41.07) (long rank test $p < 0.001$); **(D)**, Forest plot showed overall survival across patients' subgroups.

Table 5. Cox regression analysis for predicting overall survival

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age	1.015 (0.98-1.05)	0.39		
Gender: Female vs. Male	0.79 (0.46-1.38)	0.42		
Smoking: Yes vs. No	0.57 (0.29-1.11)	0.10		
Histology: Non-squamous vs. Squamous	0.49 (0.28-0.83)	0.009		
PD-L1 expression: ≥ 50% vs. < 50%	0.92 (0.36-2.33)	0.87		
Bone metastasis: Yes vs. No	2.28 (2.09-6.43)	< 0.001	2.08 (1.10-4.94)	0.030
Just before CT				
NLR1: > 5 vs. ≤ 5	8.56 (4.34-16.93)	< 0.001	8.09 (2.35-27.81)	0.001
PLR1: High vs. Low	2.89 (1.63-5.15)	< 0.001	2.81 (1.13-6.97)	0.025
RDW: High vs. Low	1.831 (1.048-3.197)	0.034		
Just before Immunotherapy				
NLR2: > 5 vs. ≤ 5	7.94 (3.99-15.78)	< 0.001		
PLR2: High vs. Low	5.08 (2.72-9.50)	< 0.001		
ΔNLR: ≥ 25% vs. < 25%	2.27 (1.32-3.89)	0.003		
ΔPLR: ≥ 25% vs. < 25%	1.83 (1.06-3.20)	0.031		

pre-treatment PLR and presence of bone metastasis in NSCLC patients treated with pembrolizumab.

Until now, many research studies have examined and evaluated the predictive and prognostic value of blood NLR and PLR in patients with various solid tumors, who received immune checkpoints inhibitors (*20,21*). The retrospective review of a recent phase 1 clinical trial reported that high baseline NLR and PLR values were linked significantly with worse OS and PFS in 90 advanced-stage cancer patients, who received treatment on an immunotherapy-based regimen (*20*). In addition, an increase in NLR and PLR values 6 weeks after baseline was associated with shorter OS and PFS.

In conjunction with other studies, our results further support the evidence that NLR (member of the markers of systemic inflammation) may predict poor response to checkpoint inhibitors and poor outcome in patients with NSCLC (*16,22,23*). However, no direct relation was found between distinct NLR cutoff values and PFS benefit (*6*). In the subsequent analysis of NLR cutoff values and OS/PFS benefit, it turned out that higher NLR cutoff was linked to a lower chance of OS benefit. Another study reports that higher cutoff value was linked with worse PFS (*24*). This suggests that the relation between NLR and prognosis could be gradual rather than a threshold one.

Recent studies report that blood neutrophils, identified by the NLR were directly linked with the number of intratumoral neutrophil populations, which may have the potential to compromise the antitumor immune response (*25,26*). Lower counts of lymphocytes usually reflect an impairment of cell-mediated immunity. It has been shown that increased infiltration of lymphocytes in the tumor region is associated with better responsiveness to treatment and prognosis in patients with solid tumors (*27*). Usually neutrophilia represents a response to systematic inflammation (*28*).

Although the biological foundation for these findings requires further elucidation, a number of recent research studies provide some explanations that should be strongly considered. It has been established that neutrophils and platelets play an important role in the development and progression of tumors as well as metastases, either by exercising a direct effect on tumor cells or by indirectly affecting other components of the tumor microenvironment. This effect is achieved through the secretion and release of various chemokines and cytokines, including transforming growth factor-beta, vascular endothelial growth factor, IL-6, IL-8 and matrix metalloproteinases (*29,30*). In addition, the latest findings of a study demonstrate that there is an association between a higher neutrophil count and decreased CD8+ content in lung cancer tumor cells (*31*), identifying neutrophilia as an inflammatory response, which suppress the antitumor immune response through inhibition of the cytotoxic activity of immune cells, in particular, that of activated CD8+T cells. Consequently, this may lower the chance of response to immunotherapy.

Consistent with the results of other research studies, we found that high PLR and presence of bone metastasis, which are common in patients with advanced NSCLC, are associated with shorter survival (*32-34*).

Several limitations were identified in our study. First of all, our study is retrospective and has a relatively small sample size. Moreover, the predictive value of NLR was not compared to new predictive markers like tumor mutational burden or microsatellite instability.

Despite these limitations, the results suggest that patients with NLR > 5 are at high risk for early progression and short overall survival. This may be helpful to clinicians in their choice of treatment, especially for patients with high pretreatment NLR - perhaps a combination of chemotherapy and immunotherapy or new molecules in clinical trials. Drugs, which are capable of transforming neutrophils into a functional state with antitumor activity, are needed in order to improve patients' outcome.

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