International Journal of Clinical Oncology and Cancer Research
2021; 6(2): 98-108
http://www.sciencepublishinggroup.com/j/ijcocr
doi: 10.11648/j.ijcocr.20210602.16
ISSN: 2578-9503 (Print); ISSN: 2578-9511 (Online)

Prognostic and Predictive Role of Neutrophil to lymphocyte Ratio in Second Line Immunotherapy of Non-small Cell Lung Cancer

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To cite this article:
Chiara Bennati, Manolo D'Arcangelo, Alessio Gili, Federica Gazzaneo, Sara Pini, Antonello Menghi, Marco Montanari, Giorgio Papiani, Valentina Mazza, Simona Scodes, Michela Spreafico, Giulio Rossi, Dora Caruso, Guido Bellezza, Martina Mandarano, Stefano Tamberi. Prognostic and Predictive Role of Neutrophil to lymphocyte Ratio in Second Line Immunotherapy of Non-small Cell Lung Cancer. *International Journal of Clinical Oncology and Cancer Research*. Vol. 6, No. 2, 2021, pp. 98-108. doi: 10.11648/j.ijcocr.20210602.16

Received: May 11, 2021; **Accepted**: May 27, 2021; **Published**: June 28, 2021

Abstract: *Background*: Programmed death-ligand 1 (PD-L1) expression at immunohistochemistry is the only approved, but still unsatisfactory, biomarker for immunotherapy in Non-Small Cell Lung Cancer (NSCLC). Neutrophil to Lymphocyte ratio (NLR) is a surrogate of systemic inflammation and could correlate with outcome to immunotherapy. This retrospective study (NCT03816657) explored the role of NLR in predicting benefit to nivolumab and susceptibility to hyperprogression (HPD). *Methods*: PD-L1, baseline and on-therapy NLR values were available in 173NSCLC patients receiving nivolumab. PD-L1 positivity was defined as expression on ≥1% of tumor cells; NLR was dichotomized in high (≥5) or low (<5). Patients were divided in 4 cohorts: 1 (PD-L1+/low NLR), 2 (PD-L1-/high NLR), 3 (PD-L1+/high NLR), 4 (PD-L1-/low NLR). A landmark analysis explored the impact of cohorts and NLR change on objective response rate (ORR), progression-free survival (PFS), overall survival (OS) and its influence on HPD. *Results*: PD-L1 was positive in 48% and negative in 52% of cases. Pre-treatment NLR was ≥5in 42% and <5 in 58%of patients; on-treatment NLR was ≥5in approximately 50% of patients. PD-L1 positivity was not associated with outcome. Both high pre- and on-therapy NLR was a negative predictor of ORR ($p=0.004$), PFS ($p<0.0001$) and OS ($p<0.0001$). High NLR cohorts (2 and 4) showed poorer outcome than low NLR cohorts. Relative NLR excursion ≥25% at 4 weeks from nivolumab start was associated with reduced PFS and OS, while its decrease or stability was associated with improved outcomes. Although NLR value and its dynamic did not influence HPD occurrence ($p=0.062$), 53% of hyperprogressors belonged to high NLR cohorts. *Conclusion*: The current retrospective analysis supports the role of high NLR as a independent negative predictive factor. Its increment during immunotherapy may identify patients with low likelihood of response to immunotherapy.

Keywords: Non-small Cell Lung Cancer, Programmed Death Ligand 1, Neutrophil to Lymphocyte Ratio, Nivolumab, Hyperprogression

1. Introduction

Over the last 5 years, treatment of advanced non-small cell lung cancer (NSCLC) with unknown driver mutations has been revolutionized by the approval of checkpoint inhibitors as single agents or in combination with chemotherapy in the first-line setting [1-5]. Although these advances we are still facing the challenge of the lack of reliable predictive biomarkers of mono-immunotherapy in the second-line scenario. Despite unanimous evidence of a trend toward improved outcome in patients with high expression of PD-L1, its role in predicting benefit is still controversial [6-9]. Recent retrospective analyses suggested a potential role of immunotherapy re-challenge leading to survival advantage, butalso in this subset of patients PD-L1 has poor predictive value [10, 11]. Among investigated biomarkers, tumor mutational burden (TMB) was the most promising but failed to predict a homogeneous survival benefit across different trials. At the moment, evidence of an association between PD-L1 expression and TMB is inconsistent [12, 13]. Another aspect of mono-immunotherapy is the controversial phenomenon of hyperprogression (HPD), a tremendous acceleration of disease growth observed in up to 30% of immunotherapy patients [14, 15]. Biological mechanisms are currently only theoretic and the prediction of susceptibility to HPD still remains elusive [16-18]. Recent studies show that pre-treatment Neutrophil to Lymphocyte ratio (NLR, quotient of absolute neutrophil and lymphocyte count) is a prognostic marker for several tumors and predictive of outcome to immunotherapy [19-21]. Although the best cut-off value has not been established, it is widely accepted that a NLR of 5 discriminates populations with different prognosis, in particular a NLR ≥ 5 is associated with the worst outcome [22-26]. Lately, attention has been focused on NLR excursion during treatment: high post-treatment values were significantly associated with poor survival in advanced cancers [27, 28].

Based on the evidence that the application of immunotherapy cannot be determined only by one predictive factor, we hypothesized that the integration of PD-L1 status and NLR could discriminate cohorts of patients with different benefit to treatment. We also explored the role of on-therapy NLR and of its evolution in influencing outcomes during immunotherapy. In order to define the difference between nivolumab and chemotherapy-induced HPD, we evaluated the incidence of this unraveling phenomenon in the frontline setting with cisplatin-based chemotherapy versus any line immunotherapy.

2. Materials and Methods

2.1. Patients

The population of this retrospective analysis (NCT03816657) included consecutive patients with advanced NSCLC who received at least one cycle of second or later line nivolumab (3mg/kg intravenously every 2 weeks) in two Italian Institutions (Hospital of Ravenna and Hospital of Perugia) between February 2015 and June 2019.

The study was approved by the local Ethical Committee (Comitato Etico della Romagna, CEROM). Patient data and laboratory results were recorded in an electronic anonymized database. Patients with squamous-cell cancers were considered as wild-type for targetable mutation, considered the low frequency of known druggable mutations in this population.

2.2. PD-L1 and NLR Assessment

PD-L1 expression was assessed on available archival tissue samples by immunohistochemistry (IHC) with clone 22C3 (monoclonal rabbit; Agilent Technologies, Santa Clara, CA) in a Ventana automated immunostainer (ULTRA, Ventana Medical Systems) according to the manufacturer's protocol and using proprietary reagents. We quoted PD-L1 positivity as expression on ≥ 1% of tumor cells. NLR value was obtained dividing the absolute neutrophil count by the lymphocyte value measured in peripheral blood at two time points: pre-treatment (within 4 weeks prior to the first infusion of nivolumab: median time 2 weeks); on-treatment (within 4 weeks after the first nivolumab infusion: median time 26 days). Based on historical evidences that a NLR ratio higher than 5 is associated with poor outcome [22-26], patients were dichotomized according to a pre-specified cut-off value of NLR as high (≥ 5) or low (< 5).

2.3. Patients Cohorts

Patients were grouped in four cohorts according to the combined PD-L1/NLR parameters: cohort 1 included PD-L1 negative and low NLR patients (N=53); cohort 2 included PD-L1 negative and high NLR (N=37); cohort 3 PD-L1 positive and low NLR (N=47) and cohort 4 PD-L1 positive and high NLR (N=36). In order to identify subgroups of patients with different sensibility to nivolumab a comparison of outcomes between the four cohorts was carried out.

2.4. Endpoints

The primary end point was overall response rate ORR calculated as the percentage of complete (CR) and partial responses (PR) among all treated patients. Response to treatment was assessed by computed tomography and classified according to RECIST 1.1 criteria [29]. Secondary outcome were overall survival (OS) and progression free survival (PFS), calculated from the start of nivolumab treatment to death and radiographic progression, respectively.

To evaluate the risk of HPD with nivolumab compared to chemotherapy, response to first line platinum doublet was collected for the same group of patients.

3. Statistical Analysis

Descriptive statistics was performed using frequencies, percentages, frequency tables for categorical variables, median and means ± standard deviation (SD) for quantitative variables. Categorical variables were evaluated by Chi-square or Fisher's exact test depending on the number of patients per group. The influence of the combined PD-L1/NLR parameters on ORR was analyzed by logistic regression approach.

Multivariate Cox proportional hazards models were used to determine whether PD-L1, NLR, combined PD-L1/NLR and other baseline characteristics were associated with PFS and OS. The Kaplan-Meier method was used to analyze survival and estimate medians with two-sided 95% confidence intervals (CI). Survival curves were compared using the log-rank test. Candidate prognostic factors with a 0.2 significance level in univariate analysis were entered in a multivariate Cox model and a backward-selection procedure was used to determine independent prognostic factor. A p-value of less than 0.05 was consider to be statistically significant. Statistical analyses were performed with STATA v. 16.1 (Stata Corp LP, College Station, TX, USA).

Table 1. *Patients' and tumor characteristics.*

Patients' and Tumor characteristics (n=173)	**n (%)**
Age (years)	
Median	65
Range	30-84
> 70	61 (35)
Sex	
Male	114 (66)
Female	59 (34)
ECOG PS	
0	85 (49)
1	75 (43)
2	12 (7)
3	1 (1)
Smoking history	
Never	24 (14)
Current Smokers	116 (67)
Former Smokers (> 1 year)	33 (19)
Histology	
Squamous	47 (27)
Non-squamous	126 (73)
Mutational Status	
EGFR	8 (9)
K-RAS	45 (29)
ALK	1 (1)
Others (BRAF, HER2, MET, ROS1)	3 (3)
Therapy Line with Nivolumab	
1	5 (3)
2	105 (61)
≥3	63 (36)
Site of metastases at diagnosis	
Lung/Pleura	91 (53)
Brain	26 (15)
Bone	51 (29)
Liver	28 (16)
Nodes	37 (21)
Others	29 (17)
Sites of progression on Nivolumab	
Lung/Pleura	91 (52)
Brain	36 (21)
Bone	47 (27)
Liver	30 (17)
Nodes	12 (7)
Others	8 (4)
Pre-treatment NLR	
≥ 5	73 (42)
< 5	100 (58)
On-treatment NLR	
≥ 5	86 (49)
< 5	87 (51)
PD-L1 status	
≥ 1%	83 (48)
< 1%	90 (52)

4. Results

4.1. Patients and Tumor Characteristics

A total of 173 patients were treated with a median of 8 cycles (range 1-37) of nivolumab. Median duration of follow-up was 7.5 months (95% CI: 0.3-94.6). Analysis of EGFR/KRAS/BRAF/HER2/MET mutations and ALK/ROS1 rearrangements were performed in all advanced adenocarcinomas. Patients and tumor characteristics are summarized in Table 1.

Median baseline and on-treatment NLR value was 4 (range, 0.5–7) and 4.9 (range, 0.9-34), respectively. Pre-treatment NLR was ≥ 5in 73 patients (42%,), on-treatment NLR was ≥ 5 in approximately 50% of patients. PD-L1 was positive (≥ 1%) in 83 cases (48%). Based on such characteristics, 53 patients (31%) were included in cohort 1 (PD-L1-/low NLR), 37 (21%) in cohort 2 (PD-L1-/high NLR), 47 (27%) in cohort 3 (PD-L1+/low NLR) and 36 (21%) in cohort 4 (PD-L1+/high NLR) (Figure 1).

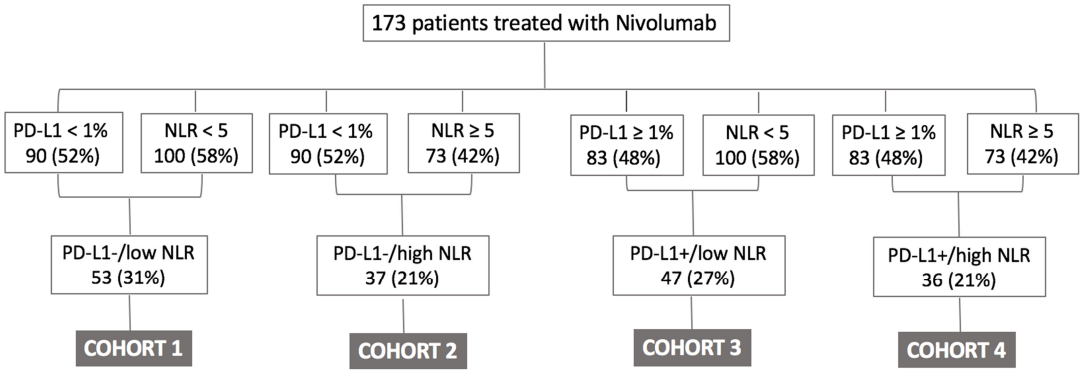

Figure 1. *Cohorts Distribution. NLR: Neutrophil to Lymphocyte ratio; PD-L1: Programmed Death Ligand 1.*

4.2. Response

ORR was 18% (31 patients; 17% and 21% in non-squamous and squamous histology, respectively), including 21 patients with ongoing responses at the time of data collection. Disease control rate (DCR, consisting of CR, PR, and stable disease - SD) was 51% (51 of 88 patients). Responses were durable (median 8.4 months, range 0.23-72.08) (Table 2). Among the 8 patients with EGFR mutation, 1 (12.5%) had a partial response. The ALK positive patient reached a 4-months

stability of disease as best response. Among the 44 patients with KRAS mutation, 5 (16%) obtained a partial response.

While response was not affected by PD-L1 status, both high baseline and on-therapy NLR significantly predicted poorer outcome: ORR in pre-treatment NLR < 5 was 25% [95% CI 17.4-34.5] vs 8.2% in NLR ≥ 5 [95% CI 3.6-17.3], p=0.004. Considering the on-treatment value of NLR, ORR was 26.4% [95% CI 18.1-36.8] in NLR < 5 vs 9.3% [CI 4.6-17.6] in NLR ≥ 5, p=0.003. In addition, low response rate to nivolumab was observed in high NLR cohorts 2 and 4 compared to low NLR cohorts 1 and 3: cohort 1 ORR 24.5% (95% CI14.6-38.1); cohort 2 ORR 10.8% (95% CI 3.9-26.1); cohort 3 ORR 25.5% (95% CI 14.8-40.2); cohort 4 ORR 5.5% (95% CI 1.3-20.4).

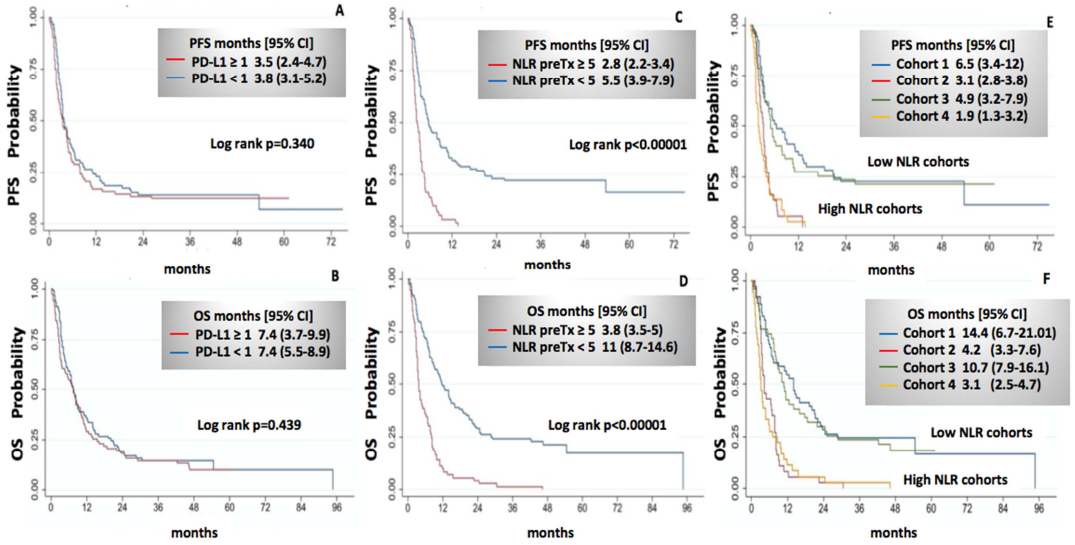

Figure 2. *PFS (A) and OS (B) probability in PD-L1<1% or ≥1%. PD-L1 positivity was not associated with outcomes; PFS (C) and OS (D) probability in baseline NLR<5 or ≥5. NLRN ≥ 5independently correlates with worse PFS and OS. PFS (E) and OS (F) probability in cohorts 1-4. High NLR cohorts significantly correlate with poorer PFS and OS.*

4.3. Survival

Median PFS was 3.5 months (95% CI 3.1-4.5months). PD-L1 positivity was not associated with PFS (p=0.340) and OS (p=0.439), while high baseline and on-treatment NLR strongly correlated with shorter PFS (p<0.0001 and p=0.015 respectively) and OS (p<0.0001 and p=0.001, respectively) (Figure 2). In the Cox regression analysis for PFS and OS the variables included were: age, sex, ECOG PS, smoking history, histology, brain/bone/liver involvement at diagnosis, mutational status, PD-L1 expression, baseline and on-therapy NLR level, and cohorts. Bone metastases at baseline (HR 1.43 [1.01-2.02], p=0.038), high pre- and on-treatment NLR (HR 2.97 [2.10-4.21], p<0.00001 and HR 2.3 [1.6-3.2], p<0.00001, respectively) and belonging to cohorts 2 and 4 (HR 2.8 [1.76-4.52], p<0.00001 and HR 3.46 [2.18-5.51], p<0.00001, respectively) significantly predicted poorer PFS. In the multivariate analysis high NLR confirmed the strong influence on PFS, irrespective of the timing of sampling (pre-therapy NLR: HR 2.30 [1.55-3.40], p<0.0001; on-therapy NLR: HR 1.59 [1.09-2.33], p 0.015).

In this heavily pretreated population median OS was 7.4 months [95% CI 5.8-8.5] with 1-year OS rate of32% [95% CI 25-39]. At the univariate analysis bone metastasis at diagnosis (HR 1.41 [1-1.99], p=0.048) and high pretreatment and on-treatment NLR (HR 3.04 [2.16-4.26], p<0.00001 and HR 2.3 [1.8-3.6], p<0.00001, respectively) predicted poorer OS. The multivariate analysis confirmed the detrimental influence of both high pre- (HR 2.30 [1.55-3.39], p<0.0001) and on-therapy NLR (HR 1.88 [1.28-2.77], p=0.001) on OS (Table 3); interestingly, this analysis showed a potential deleterious effect of immunotherapy in males vs females (HR 1.53 [1.07-2.16], p=0.017). Lastly, as confirmed by responses, high NLR cohorts 2 and 4 showed significant worsePFS and OS compared to low NLR cohorts1 and 3, irrespective of PD-L1 value (Figure 2).

4.4. Impact of NLR Excursion During Immunotherapy

Once we confirmed the negative predictive role of high NLR on the immunotherapy effectiveness, we hypothesized that its early variation from baseline could predict in advance a variable response to nivolumab, even before radiological detection. A landmark analysis was conducted to explore the effect of NLR change on ORR, PFS and OS. Excursion was defined as an arbitrary 25% increase or decrease in early NLR on-treatment value according to a 3 groups definition: group A ≥25% increase, group B no change [<25% decrease to <25% increase], group C ≥25% decrease. Sixty-nine patients were included in group A, 72 patients in group B and 32 patients in group C. Patients in group A had the worst PFS (2.9 months, 95% CI 2.3-3.5) and OS (4.6 months, 95% CI 3.4-7.4), p < 0.0001. Those in groups B and C showed similar PFS (4.6 months, 95% CI 2.2-7.7 and 5.2 months, 95% CI 3.4-7.9, respectively) and OS (8.9 months, 95% CI 5.5-12.3 and 8.9 months, 95% CI 6.4-14.1) (Figure 3).

Table 2. *Tumor response in patients with advanced NSCLC treated with nivolumab. ORR: Overall Response Rate; DCR: Disease Control Rate; DOR: Duration of response.*

Response	All Patients	p value	Non-Squamous	Squamous
	n=173		n=126	n=47
ORR, n (%) [95% CI]	31 (18) [12.8-24.4]		21 (17) [11-24.2]	10 (21) [11.6-35.6]
DCR, n (%) [95% CI]	88 (51) [43.4-58.2]		62 (49) [40.5-57.9]	26 (55) [40.6-69]
Ongoing responders, n (%)	12 (7)		11 (9)	1 (2)
DOR, median (range) months	23 (18.6-24.5)		34.2 (0.23-72.8)	3.19 (1.6-57.2)
ORR PDL1 ≥1%, (%) [95% CI]	16.8 (10.1-26.6)	P=0.729	13.1 [6.6-24.3]	27.2 [12.1-50.3]
ORR PDL1<1%, (%) [95% CI]	18.8 (12-28.4)	(ref.)	20 [11.8-31.7	16 [5.8-36.9]
ORR baseline NLR< 5, (%) [95% CI]	25 (17.4-34.5)	P=0.004	21.2 (13.5-31.7)	40 (20.4-63.4)
ORR baseline NLR ≥ 5, (%) [95% CI]	8.2 (3.6-17.3)	(ref.)	8.6 (3.2-21.4)	7.4 (1.7-26.5)
ORR on-treatment NLR < 5, (%) [95% CI]	26.4 (18.1-36.8)	P=0.003	26.1 [16.7-38.3]	27.2 [12.1-50.3]
ORR on-treatment NLR ≥ 5, (%) [95% CI]	9.3 (4.6-17.6)	(ref.)	6.5 [2.4-16.4]	16.1 [5.8-36.9]
ORR Cohort 1, (%) [95% CI]	24.5 (14.6-38.1)	-	-	-
ORR Cohort 2, (%) [95% CI]	10.8 (3.9-26.1)	-	-	-
ORR Cohort 3, (%) [95% CI]	25.5 (14.8-40.2)	-	-	-
ORR Cohort 4, (%) [95% CI]	5.5 (1.3-20.4)	-	-	-

4.5. Hyperprogression

HPD was defined as > 50% increase in tumor growth rate compared with pre-immunotherapy volume as best response at the first disease evaluation performed after starting nivolumab. HPD occurred in 40% [95% CI 32-48] of patients treated with immunotherapy and 22% [95% CI 16-29] of patients receiving an upfront platinum-doublet (p<0.001), reinforcing the idea that checkpoint inhibitors may upregulate immune modulators and the expression of oncogenic pathways differently from chemotherapy [30]. Therefore, we examined the potential influence of patients or clinical characteristics on immunotherapy and chemotherapy-related HPD. With nivolumab we observed a significant correlation of HPD with PD-L1 positivity (p=0.014), adenocarcinoma histology (p=0.006), different sites of metastatic involvement of disease at baseline (liver, p=0.0001; bone, p=0.0001; brain, p=0.001; nodes, p=0.020). Interestingly, 53% of the hyperprogressors to nivolumab (30/57 patients) belonged to high NLR cohorts (p=0.062) suggesting a potential negative influence of high pre-treatment leukocyte count imbalance on outcome to immunotherapy. In the chemotherapy arm no

specific predictive factors seem to correlate with this phenomenon (Table 4).

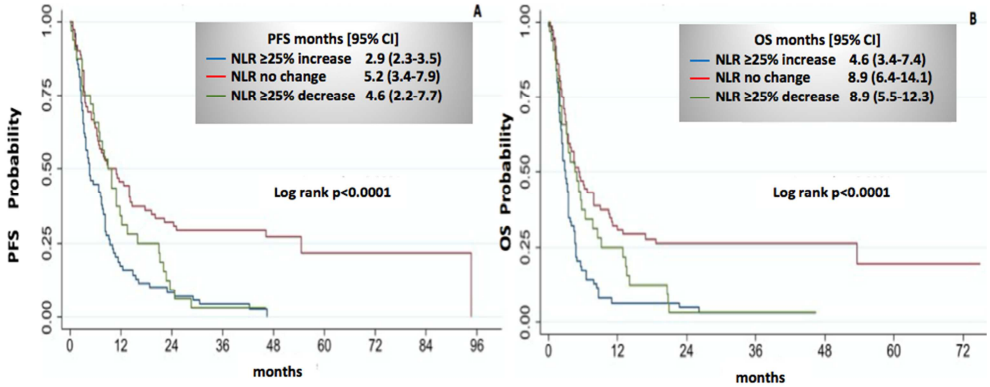

Figure 3. *PFS (A) and OS (B) probability in group 1 (≥25% NLR increase), group 2 no change (<25%NLR decrease to < 25% NLR increase), group 3 (≥25% NLR decrease).*

Table 3. *Univariate and multivariate analyses progression free survival (PFS). And of overall survival (OS) PFS: Progression Free Survival; OS: Overall Survival; ECOG PS: Eastern Cooperative Oncology Group Performance Status; C/F smokers: current/former smokers; Brain Mets: brain metastases.*

	PFS Univariate Model HR [95% CI], value	PFS Multivariate model HR [95% CI], p value	OS Univariate Model HR [95% CI], p value	OS Multivariate Model HR [95% CI], p value
Age ≤ or > 70 y	1.19 [0.85-1.66], p 0.295		1.24 [0.89-1.74], p 0.192	
Sex (Female vs Male)	1.16 [0.83-1.63], p 0.375		1.31 [0.93-1.83), p 0.116	1.53 [1.07-2.16], p 0.017
ECOG PS at Nivo start	1.14 [0.64-2.02], p 0.644		1.43 [0.80-2.55], p 0.217	
Smoking (never vs smoker)	1.48 [0.85-2.06], p 0.164		1.73 [0.99-3.02], p 0.054	
Histology (Non-Sq vs Sq)	1.26 [0.88-1.79], p 0.192		1.39 [0.98-1.99], p 0.063	
Brain mets at diagnosis (y vs n)	1.12 [0.72-1.75], p 0.602		1.08 [0.69-1.69], p 0.723	
Bone mets at diagnosis (y vs n)	1.43 [1.01-2.02], p 0.038	1.33 [0.95-1.88], p 0.095	1.41 (1-1.99), p 0.048	1.31 [0.92-1.86], p 0.130
Liver mets at diagnosis (y v n)	1.15 [0.74-1.79], p 0.512		1.02 (0.66-1.59), p 0.989	
EGFR (positive vs negative)	0.95 [0.41-2.15], p 0.903		0.79 [0.35-1.80], p 0.588	
K-RAS (positive vs negative)	1.11 [0.77-1.60], p 0.547		1.16 [0.81-1.67], p 0.400	
PD-L1 (≥ 1 vs <1)	1.16 [0.84-1.60], p 0.340		1.13 [0.82-1.55], p 0.439	
NLR baseline (≥ 5 vs <5)	2.97 [2.10-4.21], p<0.00001	2.30 [1.55-3.40], p<0.0001	3.04 [2.16-4.26], p<0.00001	2.30 [1.55-3.39], p < 0.0001
NLR on-treatment (≥ 5 vs <5)	2.3 [1.6-3.2], p<0.00001	1.59 [1.09-2.33], p 0.015	2.3 [1.8-3.6], p<0.00001	1.88 [1.28-2.77], p 0.001
Cohorts: 1 (ref)				
Cohort 2 (PD-L1-/high LNR)	2.8 [1.76-4.52], p<0.00001		3.02 [1.89-4.80], p<0.00001	
Cohort 3 (PD-L1+/low LNR)	1.10 [0.71-1.72], p 0.645		1.11 [0.71-1.73], p 0.627	
Cohort 4 (PD-L1+/high LNR)	3.46 [2.18-5.51], p<0.00001		3.33 [2.14-5.38], p<0.00001	

Table 4. *Association between HPD status and clinico-pathologic variables for Nivolumab and Platinum-doublet treated Patients with NSCLC. NSq: non squamous non-small cell lung cancer; Sq: squamous cell non-small cell lung cancer; NLR: Neutrophil to Lymphocyte ratio; PD-L1: Programmed Death Ligand 1; On-tx: on therapy; Mets: metastases.*

Hyperprogression								
	Age > 65	Gender (♂ vs ♀)	Smoking (y vs n)	NSq vs SqHistology	KRAS mut	PD-L1+	High NLR pre-Tx	High NLR on-Tx
n (%)								
Nivo any line	17 (35)	32 (35)	33/36	47 (48)	17 (45)	33 (52)	30 (45)	33 (43)
p	0.311	0.086	0.175	0.006	0.526	0.014	0.254	0.519
1st line platinum doublet	8 (16)	27 (26)	28 (26)	24 (21)	7 (17)	15 (20)	13 (20)	19 (23)
p	0.177	0.092	0.183	0.592	0.363	0.536	0.586	0.685

Table 4. *Continued.*

Hyperprogression											
	On-tx NLR ↑≥25%	NLR no change	On-tx NLR ↓≥25%	Cohort1	Cohort 2 (High NLR)	Cohort 3	Cohort 4 (High NLR)	Liver mets	Bone mets	Brain mets	Nodes mets
n (%)											
Nivo any line	29 (47)	17 (34)	11 (38)	11 (19)	16 (28)	13 (23)	17 (30)	22 (73)	35 (74)	24 (67)	9 (75)
p	0.373			0.062				0.001	0.001	0.001	0.02
1st line platinum doublet	17 (27)	15 (23)	3 (10)	12 (34)	8 (23)	10 (29)	5 (14)	7 (27)	9 (23)	6 (18)	5 (45)
p	0.201			0.8				0.812	0.984	0.771	0.155

5. Discussion

In this retrospective study we explored the role of PD-L1, NLR and their combination in predicting response to nivolumab and its influence on hyperprogression. Several studies explored the negative prognostic value of high NLR [31-44], but only few investigated NLR influence on outcome to immunotherapy in NSCLC. To our knowledge, this is the first report that has explored NLR in combination with PD-L1 expression.

Given the poor reliability of PD-L1 and the absence of clear selection criteria for second-line therapy we may run the risk of giving patients an ineffective treatment depriving them of further therapeutic chances. Merging PD-L1 with another predictor, such as NLR, may increase the chance of identifying the group of patients benefitting less from nivolumab. Although we cannot identify a unique cohort with lower response to immunotherapy, our analysis shows that all the high NLR groups (cohorts 2 and 4) derive less benefit from nivolumab.

In terms of response, NLR showed a greater weight in influencing outcome than PD-L1. Patients with low baseline and on-treatment NLR value showed a 3-fold higher ORR than subjects with high NLR, both as single parameter or in combination with PD-L1 status. Both the univariate and multivariate analyses confirmed that high NLR was an independent predictive factor of shorter PFS and OS, irrespective of the timing of blood sampling. No major clinical or pathological features seemed to influence outcome, except for sex. This aspect may be correlated to the high smoke exposure of females included in our study (median 45 packs/year in females and 35 in males). Also male were older than females (male median age 66.6 versus female 61.9), representing a more vulnerable population at higher risk of early treatment discontinuation [45, 46].

Previous retrospective reports on NLR change during immunotherapy [47-51] agree that its rapid increase is associated with worse outcome, while an early decline correlates with longer survival.

Differently from previous report by Li M. et al [49], not only patients with minimal NLR excursion during treatment but also those with a large decline from the baseline level reached the longest OS. Since NLR dynamic was mainly due to the excursion in neutrophils rather than the lymphocyte count, we may speculate that survival advantage in those with a deep NLR level decrement was primarily driven by the reduction of tumor-associated neutrophils and myeloid derived suppressor cells that are well known to have an immunosuppressive role [29]. Our findings are consistent with a previous report in metastatic renal cell carcinoma in which an early decline (≥25%) of NLR at 6-weeks was associated with an improved PFS and OS, whereas a relative increase by ≥25% was associated with poor outcomes, regardless of baseline levels [50]. Our analysis showed that the NRL excursion may be informative at 4 weeks. Since restaging in clinical practice usually occurs after a minimum of two months of treatment, a rapid NLR increment after one month of immunotherapy may suggest we are in presence of a poorly sensitive disease and lead clinicians to choose a closer radiological monitoring. This strategy could help avoiding to loose that proportion of patients defined as early progressors to immunotherapy [52]. Moreover, stabilization or reduction of NLR value may support the clinician's choice to continue treatment even in presence of a high disease burden in patients with maintenance of a stable performance status.

To date, no standardized criteria or homogeneous predictive factors are available to define susceptibility to HPD during immunotherapy. Many clinicians believe that this phenomenon is not only a prerogative of checkpoint inhibitors but it may occur also during chemotherapy linked more to the fast progressing habit of the disease than to a direct immunotherapy effect. For this reason, we investigated the rate of HPD under nivolumab in any line compared to HPD seen under chemotherapy in the same group of patients. The majority of patients received a platinum doublet as upfront therapy (97%). HPD was more common with the PD-1 inhibitor compared to mono-chemotherapy [14, 15]. Notably, this is the first retrospective study analyzing the incidence of HPD between immunotherapy and a platinum doublet. Although there's not a significant correlation between cohorts and HPD during immunotherapy, those with high baseline NLR show a trend to progress more rapidly compared to the low NLR cohorts. We also observed a strong association with PD-L1 positivity and adenocarcinoma histology, not surprising seen the pro-tumorigenic role of PD-L1 overexpression [53]. A recent large retrospective analysis by Ferrara et al. has not found a significant correlation between NLR>3 or PD-L1 status and hyperprogression [15], but the combination of these two parameters and the neutrophils excursion have never been explored. This new pattern of progression seems more likely to be dynamic and multifactorial. Furthermore, in addition to agreeing with previous observations of a significant association between HPD and disease burden [15], we could add that this phenomenon is more dependent on disease quantity rather than the site of metastasis in itself. Conversely, the lack of predictive factors potentially linked to HPD in the same patients population during first line platinum chemotherapy may corroborate the hypothesis that this phenomenon is mainly an immunotherapy prerogative while it would seem a purely random event during chemotherapy.

Although the strength of these associations is limited by sample size, the results of our study highlight the potential of identifying patients who derive less benefit from immunotherapy using a simple and inexpensive tool.

This analysis presents several limitations such as the retrospective design of the study and the limited sample size. To correctly discern the differences between immunotherapy and chemotherapy-induced HPD we should compare those patients who receive an upfront monotherapy with checkpoint inhibitors versus those who are treated exclusively with a platinum-doublet. However, at the time of analysis only few

patients were treated with a first line immunotherapy as per regulatory rules and, to date, this comparison still remains challenging as the combo chemo-immunotherapy is the standard first line approach for the majority of patients.

6. Conclusions

In conclusion, our study showed that NLR value, both at baseline and during treatment, independently correlates with ORR, PFS and OS in advanced NSCLC treated with nivolumab. In our opinion, the most important finding is that immunotherapy cannot revert the negative prognostic influence of high NLR in second or further line of treatment on NSCLC. A high excursion during immunotherapy may identify a cohort of patients with a low likelihood of response to immunotherapy. Importantly, this may help clinicians to identify early patients with low chances to respond or at higher risk of hyper-progression, who may benefit from a closer monitoring or alternative therapies. Considering this is a retrospective analysis, further studies are warranted in order to confirm prospectively these finding.

Acknowledgements

The authors are grateful to all participating patients and their families. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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