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Chronic Inflammation as a Potential Predictive Factor of Nivolumab Therapy in Non-small Cell Lung Cancer

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Abstract. *Aim: To investigate potential associations between clinical and standard peripheral blood biomarkers and clinical outcome in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. Patients and Methods: A total of 120 patients with advanced NSCLC treated at seven comprehensive cancer care centers were analyzed in this national retrospective study. Survival statistics were evaluated using the Kaplan–Meier method and Cox analysis. Results: Among clinical parameters, histology was significantly associated with progression-free survival. Univariate Cox-proportional hazards model indicated prognostic and predictive role of a panel of laboratory parameters reflecting chronic inflammatory pattern (elevated neutrophil count, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, C-reactive protein and decrease in hemoglobin and albumin). Higher serum calcium concentration was also associated with nivolumab treatment effect. Conclusion: Tumor histology was the only clinical parameter predicting the outcome of nivolumab treatment. Among the laboratory parameters, our analysis identified a laboratory panel reflecting chronic inflammation as a potential predictive marker of nivolumab treatment.*

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Nivolumab is a human monoclonal anti-programed cell death 1 (PD-1) therapy that represents a new therapeutic option in the second-line treatment of advanced non-small cell lung cancer (NSCLC). Improved efficacy and a more favorable adverse event profile have been documented for nivolumab compared to docetaxel in phase III studies. However, the objective response rate on nivolumab monotherapy is only about 20%, with the disease control rate reaching approximately 50% (1, 2). Therefore, many patients do not benefit from nivolumab treatment and, taking into account the cost/efficacy ratio, identification of predictive parameters that would aid identification of the most suitable candidates for this therapy remains a topic of high unmet medical need. Much effort has been made to demonstrate that programmed death-ligand 1 (PD-L1) expression on tumor cells represent, a potential biomarker of response to anti-PD1 therapy (3, 4). However, for nivolumab, this seems to hold true in non-squamous NSCLC only, although data for other drugs, *e.g.* pembrolizumab, have demonstrated the predictive role of PD-L1 expression even in patients with squamous histology (5). For various reasons, PD-L1 expression is still not an ideal biomarker (6). Therefore, the search for other predictive biomarkers should continue. Several approaches

in this direction include, for example, gene-signature profiles, tumor mutation burden or neoantigen expression. In addition, biomarkers of immune response are being studied, leading to introduction of new methodologies in the clinical setting (7). Finally, the potential of routinely used clinical and biochemical biomarkers is also being examined (8). The advantage of parameters based on peripheral blood cell counts, or commonly determined biochemical parameters such as C-reactive protein (CRP) or serum albumin concentration is commonly used in patients with NSCLC in daily clinical practice at minimal cost. For NSCLC, the association between these parameters and nivolumab efficacy has not been well established. The objective of the present retrospective study was to investigate the association of selected clinical, hematological and biochemical parameters with the outcome of nivolumab treatment in a multicenter national study.

Patients and Methods

Study design and treatment. Clinical and laboratory data of patients with cytologically or histologically confirmed advanced NSCLC treated with nivolumab were retrospectively analyzed. The patients were treated in the second-or higher line of treatment in an Expanded Access Program provided by Bristol-Myers Squibb at seven Oncology and Pneumo-oncology Departments in the Czech Republic in 2015 and 2016. Nivolumab was administered intravenously at the approved dose of 3 mg/kg every 2 weeks. The treatment was administered until progression (or as long as the patient benefitted from the therapy according to the treating physician) or unacceptable toxicity for a maximum of 2 years. In the case of treatment-related toxicity, corticosteroid use or interruption of nivolumab were recommended. Clinical follow-up including physical examination, chest X-ray and routine laboratory tests were performed at least every 4 weeks; computed tomography (CT) or positron-emission tomography/CT were performed at regular intervals according to the routine practice of the center or when progression was suspected based on clinical or chest X-ray examination. Clinical parameters analyzed included age, sex, Eastern Cooperative Oncology Group performance status (ECOG PS), smoking status, histology, number of previous treatment lines, the extent of the disease at the time of treatment initiation and prior radiotherapy. Laboratory parameters investigated included total neutrophil, total lymphocyte, total eosinophil and platelet counts; hemoglobin, albumin, CRP, glucose, sodium, potassium and calcium concentrations; and lactate dehydrogenase (LDH) activity were measured at the start of therapy and after 2 months of therapy, *i.e.* on the day of the first nivolumab dose and 8 weeks later (±1 week) as part of routine blood check ups. Early Access Program was approved by the local Ethics Committee and all patients gave their informed consent.

Statistical methods. Categorical variables are described using absolute and relative frequencies. Survival analysis was calculated using the Kaplan–Meier method, and all point estimates were supplemented with 95% confidence intervals (CI). Progression-free survival (PFS) was determined from the date of the initiation of nivolumab treatment to the date of the first documented radiological progression (by RECIST) (9) or death. Overall survival (OS) was determined from the date of initiation of nivolumab treatment to the date of death. The patients with no PFS or OS event were censored at the date of the last visit. Comparison of patient survival between different subgroups was performed with the log-rank test. Survival curves were plotted for all parameters with $p<0.05$.

The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were also calculated, as well as calcium corrected for albumin (Ca-corr) according to the following formula: Ca-corr=Calcium (total) + 0.020×(41.3−albumin). Any changes in laboratory parameters between the two measurements (treatment initiation and 2 months later) were also investigated.

The associations of baseline clinical characteristics and laboratory parameters with OS and PFS were analyzed. The associations between laboratory parameters and OS and PFS were assessed as continuous variables, as well as dichotomous variables using a cut-off value (as normal values and above or below normal as appropriate for the respective parameter). Univariate Cox test of proportional risks was used to calculate differences in OS and PFS for laboratory parameters as continuous variables. Point estimates of hazard ratio (HR) are shown with 95% CI. Statistical significance of HR was calculated using the Wald test. Cut-offs were determined based on the upper or lower limits of normal values for laboratory parameters. A receiver operating characteristic (ROC) analysis was calculated to estimate the optimal cut-off values for NLR, PLR and Ca-corr, using a landmark of 6 months' survival from the initiation of treatment. The optimal cut-off was selected according to the criterion of maximizing the product of sensitivity and specificity.

A risk index (RI) was calculated in patients with complete records based on biomarkers of chronic inflammation associated with PFS/OS as defined by the univariate Cox test namely: elevated leucocyte and neutrophil counts, reduced lymphocyte count, elevated NLR, reduced hemoglobin, elevated LDH and CRP. The patients were divided into three subgroups according to the number of risk factors they had: low RI: 0-2 risk factors, medium RI: 3-4 risk factors, and high RI: ≥5 risk factors. An ROC analysis was carried out to estimate the optimal cut-off values for parameters included in the RI.

A multivariate Cox proportional-hazards model was used to assess the effect of potential prognostic clinical factors, including the RI, sex, age, smoking status, histology, ECOG PS, the extent of disease at the time of treatment initiation and number of prior treatment lines on OS and PFS in patients with complete records. Hazard ratios (HR) were completed with 95% confidence intervals (CI), and the statistical significance of HR was assessed by the Wald test.

The decision on statistical significance was based on $\alpha=0.05$. Because of exploratory nature of the analysis, the Bonferroni correction was not performed.

Results

Patient characteristics. In total, 120 patients were included in the present retrospective analysis; 71 patients (59.2%) were male. The majority of patients were smokers or ex-smokers (81.7%). Eighty patients had adenocarcinoma (66.7%) and 40 squamous cell carcinoma (33.3%). The baseline patient characteristics are summarized in Table I.

Clinical parameters. Patients with adenocarcinoma had significantly shorter PFS compared to patients with squamous carcinoma [median=3.7 (95% CI=2.6-4.8) months *vs*. 6.8

Table I. *Baseline patient characteristics.*

Characteristic	n	%
Gender		
Male	71	59.2
Female	49	40.8
Smoking		
Non-smoker	22	18.3
Former smoker	43	35.8
Smoker	55	45.8
Histology		
Adenocarcinoma	80	66.7
Squamous cell carcinoma	40	33.3

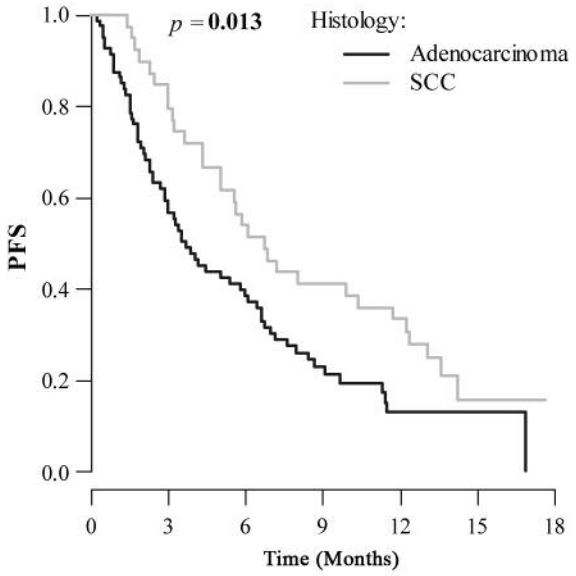

Figure 1. *Progression-free survival (PFS) after nivolumab treatment initiation according to histology. SCC: Squamous cell carcinoma.*

(95% CI 4.8-8.7) months, p=0.013]. Kaplan–Meier curves for PFS are shown in Figure 1. Sex, smoking status, age, extent of the disease at the time of treatment initiation, ECOG PS, the number of previous treatment lines and prior radiotherapy were not associated with PFS. None of the clinical parameters were associated with OS in the present study (Table II).

Laboratory parameters. Using a univariate Cox-proportional hazards model, we observed shorter PFS in patients with elevated neutrophil count, CRP and Ca-corr, and lower hemoglobin and albumin concentrations after 2 months of therapy. Shorter OS was associated with elevated neutrophil count, LDH and CRP and reduced hemoglobin at the start as well as after 2 months of therapy; and elevated NLR and platelet count at the start of therapy; elevated Ca-corr after 2 months of therapy; and reduced albumin after 2 months of therapy. The results are summarized in Table III.

When the results were evaluated based on dichotomization by limit of normal, patients with normal CRP concentration after 2 months of therapy had significantly longer PFS compared to patients with increased CRP. Improved PFS was also observed in patients with normal/increased lymphocyte count at the start of therapy, hemoglobin concentration after 2 months of therapy, Ca-corr at the start of therapy and lower CRP concentration after 2 months of therapy. Longer OS was also evident when evaluated in patients with normal/increased lymphocyte count and lower albumin and sodium concentrations at the start of therapy. When evaluated after 2 months of therapy, significantly improved OS was observed in patients with normal/lower neutrophil count, LDH activity and CRP concentration, and normal/increased hemoglobin. Higher NLR (cut-off value 3.8) and PLR (cut-off value 169.1) were significantly associated with lower OS, but not with PFS. Ca-corr under the cut-off value at the start of and after 2 months of therapy correlated with shorter OS and with shorter PFS when measured 2 months into therapy. The results are summarized in Table IV and Kaplan-Meier curves for statistically significant results are shown in Figure 2.

When changes to laboratory parameters between the two measurements were investigated, only patients with an increase in eosinophil count had significantly better OS compared to patients with stable or reduced counts. The results are summarized in Table V.

When the patients were divided into three subgroups according to the RI, statistically significant differences were observed both in PFS and OS at the time of treatment initiation and also after 2 months of therapy. Higher RI was associated with shorter PFS and OS. The results are summarized in Table VI and Kaplan–Meier curves for statistically significant results are shown in Figure 3.

Multivariate Cox proportional-hazard model. In multivariate Cox proportional-hazard model, histology was confirmed as the only significant predictive clinical parameter for OS. OS was shorter in patients with adenocarcinoma compared to patients with squamous cell carcinoma (HR=2.23; p=0.045). None of the clinical parameters was associated with PFS. RI was associated with OS and also with PFS in patients with high RI (≥5 factors; HR=6.00 and 2.20, p<0.001 and p=0.034, respectively) compared to patients with low RI (≤2 risk factors; Table VI).

Discussion

The data from the present retrospective analysis identified a number of potential predictors of outcome in patients with NSCLC treated with nivolumab in second and higher lines

Table II. *Overall (OS) and progression-free (PFS) survival after nivolumab treatment initiation according to baseline characteristics.*

Characteristic	n	Median OS (95% CI)	*p*-Value*	Median PFS (95% CI)	*p*-Value*
Gender					
Male	71	11.2 (9.0-13.4)	0.188	5.7 (4.2-7.2)	0.747
Female	49	8.0 (1.7-14.2)		3.7 (1.9-5.4)	
Smoking					
Non-smoker	22	4.9 (0.1-10.5)	0.144	3.0 (2.0-4.0)	0.178
Former smoker	43	9.2 (6.5-12.0)		5.4 (3.3-7.5)	
Smoker	55	12.0 (10.3-13.7)		5.9 (3.7-8.0)	
Histology					
Adenocarcinoma	80	9.2 (5.7-12.8)	0.329	3.7 (2.6-4.8)	0.013
Squamous cell carcinoma	40	11.2 (9.5-12.9)		6.8 (4.8-8.7)	
Radiotherapy before nivolumab treatment**					
Yes	21	7.3 (1.7-13.0)	0.126	5.8 (2.6-9.0)	0.399
No	63	10.6 (7.4-13.8)		3.7 (1.7-5.6)	
Age at initiation of nivolumab treatment					
≤65 Years	53	9.9 (7.3-12.6)	0.411	4.1 (2.3-6.0)	0.229
>65 Years	67	11.2 (8.3-14.1)		6.0 (4.0-8.0)	
Extent of the disease at treatment initiation					
III A or III B	16	12.6 (11.6-13.6)	0.132	6.6 (0.7-12.6)	0.074
IV	104	9.9 (7.3-12.5)		5.0 (3.1-6.9)	
ECOG PS at initiation of nivolumab treatment					
0	30	10.9 (8.4-13.4)	0.416	6.1 (2.2-9.9)	0.776
1	90	10.6 (7.5-13.7)		5.0 (3.0-7.0)	
Number of prior treatment lines					
1	47	10.7 (8.8-12.6)	0.609	5.0 (2.4-7.6)	0.948
2	36	11.7 (7.8-15.7)		5.4 (3.5-7.3)	
3 or more	37	10.6 (5.8-15.4)		4.1 (0.9-7.4)	

CI: Confidence intervaI; ECOG PS: Eastern Cooperative Oncology Group performance status. *Log-rank test. **Only 84 patients had valid record for radiotherapy before nivolumab treatment.

of therapy. The tumor histology was a significant clinical predictor of outcome in the multivariate analysis of the present cohort of patients. Our proposed RI based on parameters associated with chronic inflammation may also be suggested for future prospective studies.

The results of previous studies examining different clinical parameters as potential predictors of immunotherapy outcome are inconsistent with regard to their results as well as regarding the studied tumor types, therapies and patient populations (10-16). In the present study, only histology was significantly associated with PFS. Studies in melanoma indicated a possible influence of ECOG PS, sex and age on OS and objective response rate in patients treated with PD1 inhibitors (10,11). Older age might possibly be connected with increased levels of tumor-suppressive cells and female sex with improved function of T-helper cells (10). However, these results were not replicated in the present study. Some smaller studies suggest that histology (better response in patients with squamous histology) and worse performance (ECOG PS≥2) may affect nivolumab efficacy (12-16). Our analysis as well as some other studies did not confirm the effect of ECOG PS on survival (15-17). Although ECOG PS is a well-known prognostic factor for NSCLC, the present cohort did not include patients with PS >1, *i.e.* patients with significantly worse prognosis. Thus, only further data in patients with wider range of PS would address the predictive role of this parameter. However, it may be difficult to offer anti-PD1 therapy to patients with poor PS and this group of patients are usually highly selected with PS≥2.

Better results for nivolumab observed in squamous cell NSCLC might be partly explained by higher mutational burden/neoantigen expression and consequently greater immunogenicity of these tumors (18). The present data also support this notion. Smoking history is another frequently discussed predictive parameter associated with increased mutational load of the tumor (18). Three smaller studies examining the predictive role of smoking history reported contradictory results (12, 14, 16). However, meta-analysis of large clinical trials with checkpoint inhibitors indicates that a history of smoking is associated with the efficacy of immunotherapy (17). In the present study, only non-significant numerical differences indicating better PFS and OS in smokers were noted in the univariate analysis. This might be explained by the limited number of patients, in particular never smokers, in the present analysis.

Table III. *Overall and progression-free survival results according to laboratory parameters using a univariable Cox proportional-hazards model*.*

Laboratory parameter	Time point	N	Overall survival		Progression-free survival	
			HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Neutrophil count**	Initial	120	1.094 (1.008-1.186)	0.032	1.033 (0.964-1.108)	0.359
	2 Months	106	1.210 (1.104-1.327)	<0.001	1.098 (1.019-1.182)	0.014
Lymphocyte count**	Initial	120	0.799 (0.561-1.138)	0.214	0.946 (0.710-1.262)	0.706
	2 Months	106	0.572 (0.387-0.844)	0.005	0.855 (0.635-1.150)	0.300
Eosinophil count**	Initial	120	0.833 (0.224-3.094)	0.785	1.329 (0.576-3.063)	0.505
	2 Months	106	0.346 (0.094-1.273)	0.110	0.815 (0.473-1.405)	0.462
Hemoglobin	Initial	120	0.980 (0.968-0.992)	0.001	0.990 (0.980-1.000)	0.054
	2 Months	108	0.969 (0.954-0.985)	<0.001	0.976 (0.963-0.990)	0.001
Platelet count**	Initial	119	1.003 (1.000-1.005)	0.023	1.001 (0.999-1.003)	0.309
	2 Months	108	1.002 (1.000-1.005)	0.064	1.001 (0.999-1.003)	0.227
Albumin	Initial	86	0.939 (0.875-1.008)	0.084	0.950 (0.893-1.011)	0.105
	2 Months	71	0.886 (0.819-0.958)	0.003	0.929 (0.869-0.992)	0.029
LDH	Initial	70	1.382 (1.101-1.736)	0.005	1.200 (0.978-1.471)	0.080
	2 Months	64	1.249 (1.093-1.427)	0.001	1.127 (0.995-1.276)	0.060
CRP	Initial	93	1.013 (1.006-1.021)	<0.001	1.008 (1.002-1.015)	0.010
	2 Months	81	1.007 (1.003-1.012)	0.001	1.005 (1.001-1.009)	0.011
Glucose	Initial	98	0.947 (0.818-1.097)	0.468	0.969 (0.859-1.093)	0.610
	2 Months	90	0.942 (0.793-1.120)	0.500	0.995 (0.870-1.137)	0.936
Sodium	Initial	119	0.933 (0.869-1.001)	0.054	0.986 (0.926-1.049)	0.653
	2 Months	108	0.928 (0.843-1.022)	0.130	0.990 (0.914-1.072)	0.800
Potassium	Initial	119	1.214 (0.653-2.254)	0.540	0.949 (0.580-1.553)	0.835
	2 Months	108	0.859 (0.465-1.586)	0.627	1.044 (0.631-1.728)	0.868
Ca-corr	Initial	67	1.179 (0.918-1.513)	0.197	1.004 (0.818-1.232)	0.967
	2 Months	45	1.415 (1.145-1.749)	0.001	1.428 (1.133-1.801)	0.003
NLR	Initial	120	1.044 (1.001-1.088)	0.043	1.033 (0.985-1.083)	0.185
PLR	Initial	119	1.001 (1.000-1.001)	0.134	1.000 (0.999-1.001)	0.663

Ca-corr: Corrected calcium; CI: confidence intervaI; CRP: C-reactive protein; LDH: lactate dehydrogenase; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio. *Change of 0.1 was considered in the Cox model, continuous data were used. **Absolute.

Recent reports indicate potential synergy between radiotherapy and nivolumab (19). In the present retrospective analysis, information on radiotherapy was available in only two-thirds of patients and there was also a considerably different time between the administration of radiotherapy and the start of treatment with nivolumab. Therefore, it is not possible to come to a conclusion regarding any association between nivolumab treatment and the efficacy of radiotherapy. However, future studies should take into account the potential association between the end of radiotherapy and the onset of immunotherapy. The present data do indicate that nivolumab may be equally effective in elderly patients and in higher lines of therapy.

Biomarkers play an essential role in the management of patients with cancer (20). The present study follows similar investigations carried out in metastatic melanoma that represents, in many aspects, a model tumor for immunotherapy (10, 21). An indisputable advantage of biomarkers derived from peripheral blood cell counts is the practicality of routine use in contrast to expensive and laborious approaches such as the determination of the tumor mutational load (22).

Laboratory parameters, as possible predictive biomarkers of checkpoint inhibitors, were first investigated in patients with melanoma treated with ipilimumab and nivolumab. LDH, CRP and leukocyte counts (including eosinophils, lymphocytes and neutrophils) were suggested as potential biomarkers (10, 11, 21, 23, 24). An association of LDH activity, CRP concentration and neutrophil counts with the efficacy of nivolumab might be explained by the tumor burden and potential effect on the function of tumor-infiltrating lymphocytes (25). These biomarkers were also investigated in patients with NSCLC. LDH, CRP, NLR, PLR, and neutrophil, lymphocyte and eosinophil counts have been associated with both OS and sometimes also with PFS in these studies (8, 12, 15, 16). However, the results between the studies were inconsistent. This might be caused by, on the one hand, often smaller sets of patients with retrospective data, but also by the different cut-offs used in these studies. The data indicated that the use of different cut-offs or the timing of measurement at the start or during the therapy may affect the results (11, 12, 24). Therefore, in the present study we decided to use continuous variables for laboratory

Table IV. *Overall (OS) and progression-free (PFS) survival after nivolumab treatment initiation according to laboratory parameters (only statistically significant results are shown).*

Laboratory parameter	Timepoint	Value	N	Median OS (95% CI), months	*p*-Value*	Median PFS (95% CI), months	*p*-Value*
Neutrophil count	2 Months	≤Normal	72	12.2 (9.9-14.5)	0.013	6.8 (5.3-8.2)	0.057
		>Normal	34	7.5 (2.2-12.7)		4.1 (2.1-6.2)	
Lymphocyte count	Initial	<Normal	19	5.9 (1.0-10.8)	0.003	3.2 (2.7-3.8)	0.030
		≥Normal	101	12.0 (9.7-14.4)		5.8 (4.0-7.6)	
Hemoglobin	2 Months	<Normal	63	9.7 (6.7-12.8)	0.006	5.4 (3.4-7.4)	0.024
		Normal	45	14.6 (11.6-17.6)		8.4 (5.8-11.0)	
Albumin	Initial	<Normal	10	3.2 (1.1-5.3)	0.001	2.9 (0.6-5.2)	0.050
		≥Normal	76	11.7 (9.7-13.8)		5.9 (3.6-8.1)	
LDH	2 Months	≤Normal	35	14.6 (11.2-18.0)	0.010	6.1 (3.8-8.3)	0.197
		>Normal	29	9.7 (6.1-13.4)		5.8 (0.3-11.3)	
CRP	2 Months	≤Normal	20	14.6 (12.4-16.8)	0.021	9.9 (3.9-15.9)	0.007
		>Normal	61	10.6 (7.8-13.3)		4.4 (2.7-6.0)	
Sodium	Initial	< Normal	11	5.2 (0.1-11.9)	0.022	2.4 (0.9-4.0)	0.129
		≥Normal	108	11.2 (8.5-13.9)		5.7 (4.2-7.1)	
Ca-corr	Initial	≤2.40 mmol/l	38	13.1 (9.8-16.4)	0.017	5.0 (2.5-7.5)	0.435
		>2.40 mmol/l	29	7.9 (1.8-14.0)		4.4 (0.3-8.4)	
	2 Months	≤2.39 mmol/l	26	13.1 (9.7-16.4)	0.001	7.0 (4.4-9.5)	0.014
		>2.39 mmol/l	19	5.7 (2.7-8.6)		3.3 (2.3-4.3)	
NLR	Initial	≤3.8	60	14.2 (10.8-17.7)	0.020	6.1 (3.9-8.2)	0.321
		>3.8	60	9.2 (6.3-12.2)		4.1 (2.5-5.6)	
PLR	Initial	≤169.1	48	14.2 (10.7-17.7)	0.014	6.6 (4.7-8.6)	0.108
		>169.1	71	9.2 (6.4-12.0)		3.9 (2.1-5.6)	

Ca-corr: Corrected calcium; CI: confidence intervaI; CRP: C-reactive protein; LDH: lactate dehydrogenase; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio.*Log-rank test.

Table V. *Overall (OS) and progression-free (PFS) survival after nivolumab treatment initiation according to laboratory parameters (only statistically significant results are shown).*

Laboratory parameter	N	Median OS (95% CI), months	*p*-Value*	Median PFS (95% CI), months	*p*-Value*
Eosinophil count					
Decrease/constant	45	9.7 (6.0-13.4)	0.029	5.9 (3.2-8.5)	0.929
Increase	61	12.6 (9.9-15.4)		6.0 (4.5-7.4)	

CI: Confidence intervaI. *Log-rank test.

parameters and evaluated the laboratory parameters at the start and after 2 months of treatment (which is a time potentially associated with the radiologically proven relationship to the treatment response prognosis) (26). Our results indicate the possible influence of neutrophils, lymphocytes, NLR, LDH and CRP on PFS or OS. We also assessed these parameters based on cut-offs (under or above normal values) that are easily transferred to other studies and clinical practice. In general, our results verify the possible impact of neutrophils, lymphocytes, LDH and CRP on prognosis or prediction of nivolumab effect. We registered only one statistically significant effect in results that described changes of laboratory parameters, namely changes in eosinophils count, that probably reflect the low relevance of this measurement in clinical practice.

In addition to peripheral blood cell counts, peripheral blood cell count-derived ratios, CRP, LDH and other potential laboratory biomarkers were also evaluated in the present study. Albumin, a negative acute-phase serum protein, is a prognostic biomarker in many solid tumors, including NSCLC (27). Prognostic significance of serum albumin was observed in this study. Moreover, a possible influence of serum albumin on PFS was also noted. Low hemoglobin and hypercalcemia have been found to be associated with poor prognosis across a range of solid tumors (28). Moreover, calcium signaling is also important for immune response (29). In the present study,

Table VI. *Multivariable Cox proportional-hazards model for overall and progression-free survival.*

Characteristic	n	Overall survival		Progression-free survival	
		HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Number of risk factors					
0-2	22	1.00	-	1.00	-
3-4	22	2.25 (0.80-6.29)	0.123	0.75 (0.32-1.78)	0.521
≥5	33	6.00 (2.36-15.28)	<0.001	2.20 (1.06-4.56)	0.034
Gender					
Female	32	1.00	-	1.00	-
Male	45	0.65 (0.30-1.41)	0.278	0.80 (0.41-1.57)	0.512
Smoking					
Non smoker	16	1.00	-	1.00	-
Former smoker	25	0.96 (0.35-2.65)	0.933	1.41 (0.59-3.37)	0.436
Smoker	36	1.16 (0.42-3.22)	0.771	1.58 (0.63-4.01)	0.331
Histology					
Squamous cell carcinoma	31	1.00	-	1.00	-
Adenocarcinoma	46	2.23 (1.02-4.91)	0.045	1.80 (0.89-3.64)	0.100
Age at initiation of nivolumab treatment					
≤65 Years	29	1.00	-	1.00	-
>65 Years	48	0.66 (0.32-1.35)	0.252	1.16 (0.62-2.18)	0.647
ECOG PS at initiation of nivolumab treatment					
0	14	1.00	-	1.00	-
1	63	1.19 (0.46-3.08)	0.727	0.78 (0.36-1.70)	0.530
Extent of disease at treatment initiation					
III A or III B	12	1.00	-	1.00	-
IV	65	2.61 (0.94-7.25)	0.065	2.28 (0.93-5.60)	0.072
Number of previous treatment lines					
1	26	1.00	-	1.00	-
2	21	0.43 (0.16-1.18)	0.102	0.81 (0.39-1.70)	0.580
≥3	30	1.20 (0.59-2.45)	0.616	1.23 (0.63-2.39)	0.545

ECOG PS: Eastern Cooperative Oncology Group performance status.

a significantly shorter PFS and OS was evident in patients with lower hemoglobin concentration, with the difference being most marked after 2 months of nivolumab treatment. A lower calcium level corrected for albumin (due to possible impact of albumin on effective calcium concentration) was associated with significantly longer OS and PFS after 2 months of treatment. Elevated potassium may have a negative effect on lymphocyte function (30), but potassium concentration did not affect the efficacy of nivolumab in the present study. A low sodium level is a negative prognostic factor in some tumor types, including NSCLC (31). In the present study, sodium concentration was associated with OS using data with a cut-off, but not in continuous variable data set. Therefore, it is possible that concentrations have no effect on OS above a certain limit (negative result of sodium evaluation as a continuous variable), but only below a certain threshold (positive result using a cut-off value). Hypoglycemia may potentially reduce lymphocyte function (25). In contrast, another study indicated a possible negative effect of hyperglycemia associated with chronic tumor inflammation (32). Thus, the effect, if any, of blood glucose on the efficacy of nivolumab treatment is unclear. Our study did not demonstrate any association of glucose with PFS or OS.

The immune response and inflammatory reaction elicited by the tumor growth can result, depending on context, in both suppression and stimulation of tumor growth. During the past decade, much effort has been focused on the identification of biomarkers that would reflect this delicate balance of host response to tumor growth. Because repeated measurements of peripheral blood cell counts are available for virtually all patients, simple indices derived from these, such as NLR or PLR, have been introduced in retrospective studies. It has been demonstrated in individual studies as well as in meta-analyses that these peripheral blood cell count-derived ratios predict prognosis across a range of solid tumors (33-35). However, this approach has certain limitations for multicentric studies as different methods of differential count determination may result in significant differences in peripheral blood cell count-derived ratios (36). The present study also showed an effect of NLR on prognosis of patients treated with nivolumab. Although we observed an association between platelet increase and OS in continuous variable analysis, there was no

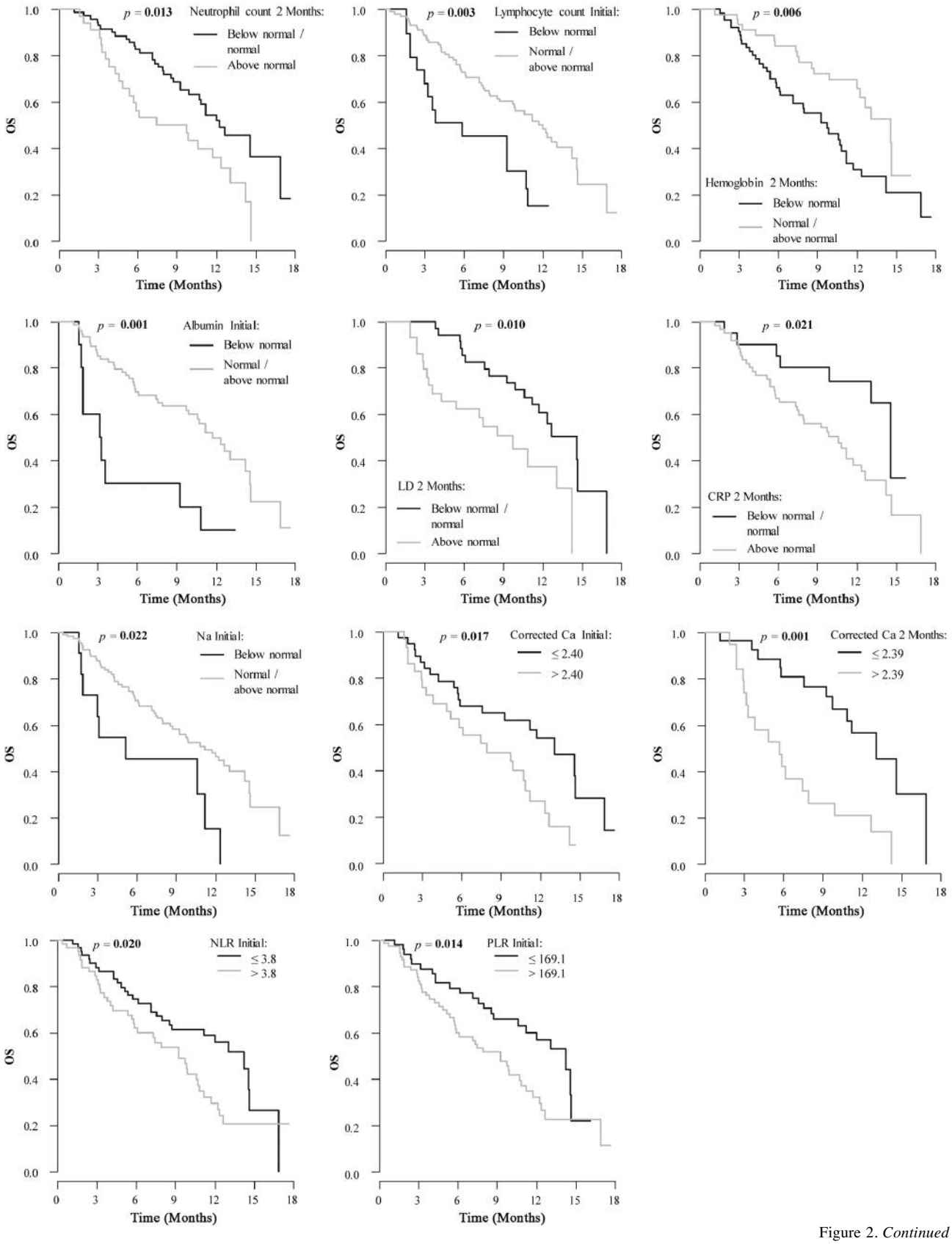

Figure 2. *Continued*

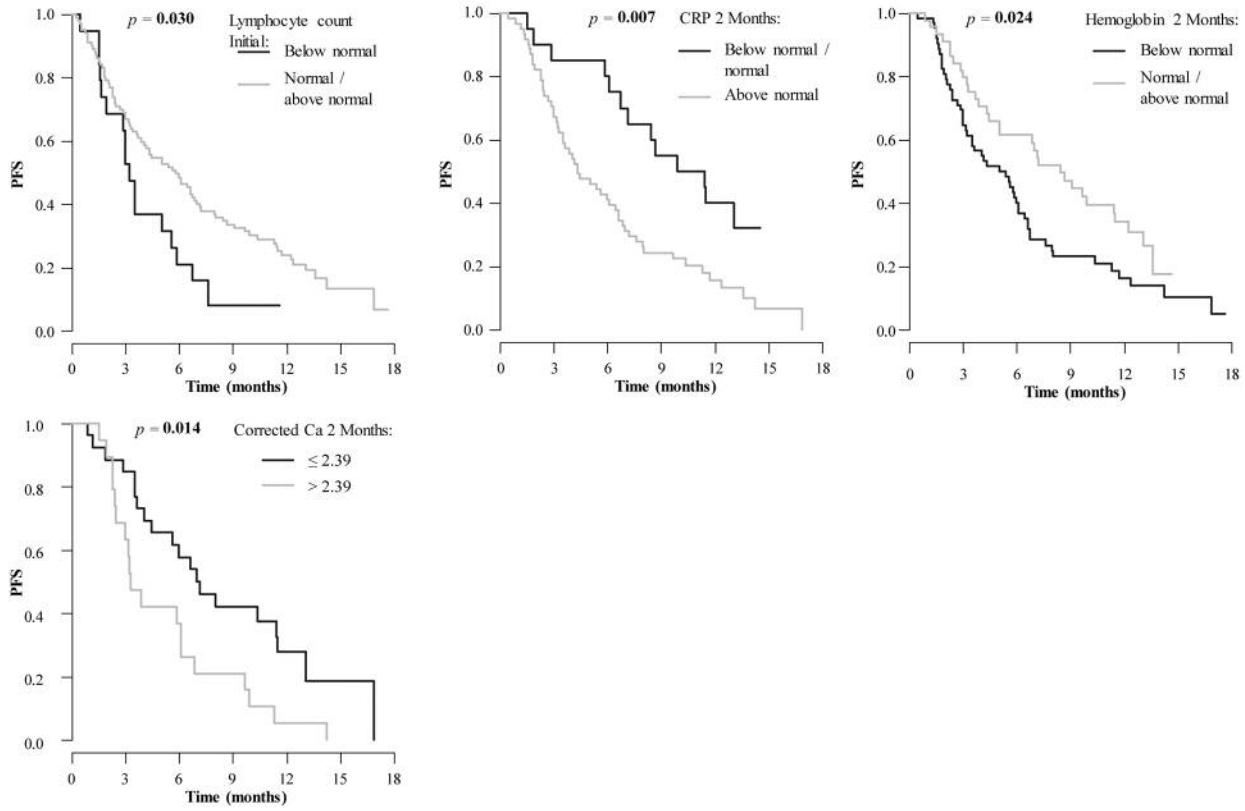

Figure 2. *Overall (OS) and progression-free (PFS) survival after nivolumab treatment initiation according to selected laboratory parameters.*

effect of PLR on patient prognosis. This is inconsistent with the results when using cut-off values, where increased PLR was associated with shorter OS. More data are, therefore, needed to clarify these discrepancies.

Potential predictive role of an index reflecting chronic inflammation pattern on PFS and OS was examined based on a data set of single laboratory markers. Chronic inflammation is one of the hallmarks of cancer and has a negative impact on patient prognosis (37, 38). This inflammatory response may potentially interfere with the effects of immunotherapy (39). The laboratory parameters associated with OS or PFS in the present study reflect molecular pathways of chronic inflammation and were shown to predict prognosis across a range of different tumors (40-45). In the present study, the impact of an index consisting of increased neutrophil count, reduced lymphocyte count, higher NLR, reduced hemoglobin and albumin, and elevated LDH and CRP on the outcome of nivolumab treatment was investigated. We observed a statistically significant effect of this RI on OS and also a significant effect on PFS in both univariate and multivariate analysis.

The present study has several limitations. Firstly, it was a retrospective study of patients from an Extended Access Program that may be biased with regard to patient selection. Secondly, the majority of patients were not investigated for PD-L1 status (due to no necessity to test the PD-L1 status before using nivolumab), potentially also influencing treatment results for some patients, although at least in patients with squamous cell NSCLC, PD-L1 expression does not predict the efficacy of nivolumab (1). Finally, laboratory parameter data were incomplete and some analyses lacked sufficient statistical power. Thus, the present report should be regarded as exploratory and the results should be verified in a larger prospective study.

In conclusion, in the present retrospective exploratory analysis, tumor histology was the only clinical parameter predicting the outcome of nivolumab treatment. Among the laboratory parameters, the results suggest a potential role for a panel combining selected laboratory parameters that reflect chronic inflammation. The corrected calcium may also affect the efficacy of nivolumab treatment.

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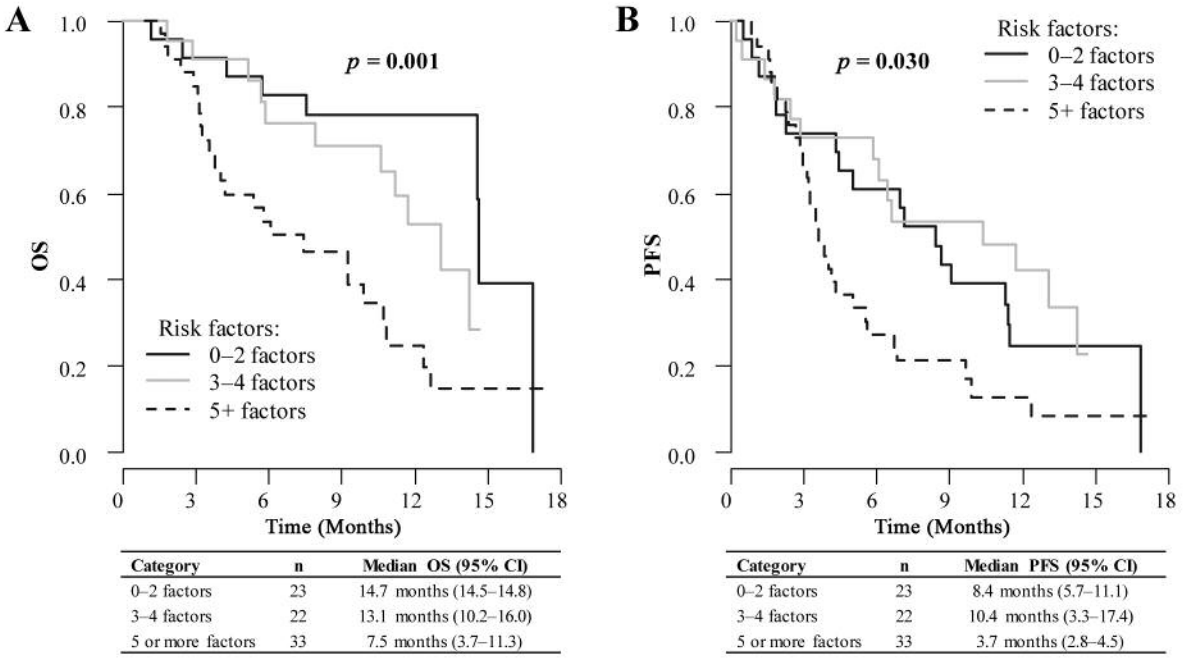

Category	n	Median OS (95% CI)
0–2 factors	23	14.7 months (14.5–14.8)
3–4 factors	22	13.1 months (10.2–16.0)
5 or more factors	33	7.5 months (3.7–11.3)

Category	n	Median PFS (95% CI)
0–2 factors	23	8.4 months (5.7–11.1)
3–4 factors	22	10.4 months (3.3–17.4)
5 or more factors	33	3.7 months (2.8–4.5)

Category	n	Median OS (95% CI)
0–2 factors	33	14.7 months (12.6–16.7)
3–4 factors	18	10.6 months (6.4–14.8)
5 or more factors	17	5.7 months (2.1–9.3)

Category	n	Median PFS (95% CI)
0–2 factors	33	9.1 months (5.3–13.0)
3–4 factors	18	6.1 months (4.4–7.7)
5 or more factors	17	3.2 months (2.1–4.3)

Figure 3. *Overall (OS; A and C) and progression-free (PFS; B and D) survival from initiation of nivolumab treatment according to number of risk factors at nivolumab initiation (A and B) and after 2 months of treatment (C and D).*

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