ANTICANCER RESEARCH *41*: 1599-1606 (2021)
doi:10.21873/anticanres.14921

Blood Cell Count Biomarkers Predicting Efficacy of Pembrolizumab as Second-line Therapy for Advanced Urothelial Carcinoma

YOSHIFUMI KADONO[1], SHOHEI KAWAGUCHI[1], TAKAHIRO NOHARA[1], KAZUYOSHI SHIGEHARA[1], KOUJI IZUMI[1], TAIKI KAMIJIMA[2], CHIKASHI SETO[2], AKINOBU TAKANO[3], SATOSHI YOTSUYANAGI[3], RYUNOSUKE NAKAGAWA[4], TOHRU MIYAGI[4], SHUHEI AOYAMA[5], HIDEKI ASAHI[6], RIE FUKUDA[7] and ATSUSHI MIZOKAMI[1]

[1]*Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan;*
[2]*Department of Urology, Toyama Prefectural Central Hospital, Toyama, Japan;*
[3]*Department of Urology, Kouseiren Takaoka Hospital, Takaoka, Japan;*
[4]*Department of Urology, Ishikawa Prefectural Central Hospital, Kanazawa, Japan;*
[5]*Department of Urology, Noto General Hospital, Nanao, Japan;*
[6]*Department of Urology, Kaga Medical Center, Kaga, Japan;*
[7]*Department of Urology, Fukui-ken Saiseikai Hospital, Fukui, Japan*

Abstract. *Background/Aim: To investigate the blood markers for predicting pembrolizumab efficacy in advanced urothelial carcinoma (UC). Patients and Methods: This study included 91 advanced UC patients. The relationship between prognosis and markers from peripheral blood cell counts, including the neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), monocyte–lymphocyte ratio (MLR), and systemic inflammation response index (SIRI=monocytes × neutrophils/lymphocytes), was evaluated. Results: Multivariate analysis indicated that pretreatment NLR and the 1-month-change NLR were both significantly associated with overall survival (OS) after pembrolizumab initiation. When the patients were divided into four groups according to calculated cutoffs using Cox proportional hazard model, the pretreatment NLR <2.9 and 1-month change NLR <+43% groups had a significantly better OS than the pretreatment NLR ≥2.9 and 1-month-change NLR ≥+43% groups. Conclusion: NLR, MLR, PLR and SIRI before pembrolizumab and 1-month-change NLR in advanced UC correlated with OS after pembrolizumab treatment.*

Correspondence to: Yoshifumi Kadono, MD, Ph.D., Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduate School of Medical Science, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8640, Japan. Tel: +81 762652393, e-mail: yskadono@yahoo.co.jp

Key Words: Urothelial carcinoma, pembrolizumab, neutrophil–lymphocyte ratio, monocyte, platelet.

Until recently, there was no effective second-line treatment for advanced urothelial carcinoma (UC) after failed chemotherapy. In a phase III randomized study (KEYNOTE-045) on second-line treatment for advanced UC patients after failure of platinum-based chemotherapy, pembrolizumab resulted in a longer overall survival (OS) (10.3 months *vs.* 7.4 months) and achieved a 27% risk reduction in death than the existing second-line chemotherapy (1). As a result, pembrolizumab is currently considered the standard-of-care for second-line treatment for advanced UC. However, the biomarkers that predict the efficacy of this treatment are not clear.

Tumor-promoting inflammation is one of the hallmarks of cancer that contributes to tumorigenesis and the development of cancer (2). In peripheral blood, neutrophils, lymphocytes, and monocytes are largely responsible for an inflammatory response. Accumulating evidence has revealed that these inflammatory markers can be an important indicator of cancer progression. In the field of urology, certain peripheral blood cell count markers, including the neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), monocyte–lymphocyte ratio (MLR), and systemic inflammation response index (SIRI), have been reported to have an impact on prognosis (3-6). There are reports that the NLR before immune checkpoint inhibitor (ICI) treatment is effective in predicting the efficacy of treatment (7), and that changes in the NLR after ICI are effective in predicting prognosis (8, 9).

The NLR, PLR, MLR, and SIRI can be easily calculated using peripheral blood cell count. However, the relationship

between these factors and the therapeutic effects of ICI have not been adequately reported. In this study, we retrospectively investigated the blood markers related to pembrolizumab second-line treatment in advanced UC patients.

Patients and Methods

Patients. We retrospectively evaluated consecutive advanced and recurrent UC patients who were treated with pembrolizumab, after progression following platinum-based chemotherapy, at Kanazawa University and related hospitals from January 2018 to October 2019. All patients had histologically or cytologically confirmed UC of the renal pelvis, ureter, or bladder. Patients with a full set of blood data before and at approximately 1 month (from 12 to 42 days) after pembrolizumab commencement, without an active infection accompanied by fever, and those without chronic inflammatory or autoimmune disease at least 1.5 months after pembrolizumab commencement, were included in this study.

Treatment and follow-up examination. Pembrolizumab was administered at a dose of 200 mg every three weeks until discontinuation due to disease progression or the occurrence of serious adverse events. We obtained patient medical records, from which the clinical features, including age, sex, primary tumor site, histological findings, metastatic organs, duration since previous chemotherapy, and Eastern Cooperative Oncology Group Scale of Performance Status (ECOG-PS), were recorded. Laboratory data, including white blood cell count (WBC, /ml), hemoglobin (Hb, mg/dl) level, platelet count (/ml), neutrophil counts (/ml), lymphocyte counts (/ml), and monocyte counts (/ml), before the commencement of treatment and after 1 month, were collected from the patient medical records. SIRI and 1-month-change NLR (%) were calculated according to the following formula: SIRI=monocytes × neutrophils/lymphocytes, 1-month-change NLR (%)=[(NLR at 1 month/pretreatment NLR) - 1] × 100. The 1-month-change MLR, 1-month-change SIRI, and 1-month-change PLR were calculated in the same way as the 1-month-change NLR. The observation period and OS were calculated from the commencement of pembrolizumab to death or last follow-up.

Statistical analysis. The incidence and percentage of each factor were calculated using categorical variables, and all continuous variables are reported as the median and range. OS was estimated using Kaplan–Meier methods, and differences in OS were evaluated using the log-rank test. Cox proportional-hazards models were used to identify the prognostic factors for OS. Correlations between variables were calculated using Spearman's rank correlation, and the correlation coefficients (CC) were then interpreted according to Cohen's guidelines for correlation effect sizes, which define a small correlation as 0.1-0.3, medium as 0.3-0.5, and large as 0.5-1.0 (10). To determine the cutoff points of NLR before pembrolizumab initiation and the 1-month change NLR for to predict OS, the hazard ratio (HR) and *p*-value at each cutoff point calculated using Cox proportional hazard model were plotted; thereby, each cutoff point was set as the number with highest HR and lowest p-value. All data analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA), and a *p*-value of <0.05 was used to indicate statistical significance.

Table I. *Demographics of the study population*.

Variable	Median (range) or n (%)
Total number of patients	91
Age, years	73 (48 to 85)
Male/Female	65 (71)/26 (29)
Primary tumor site, upper urinary tract	39 (43)/52 (57)
Pure UC in histologic testing	80 (88)/11 (12)
Surgical treatment	43 (47)/48 (53)
ECOG PS, 0-1/2-4	74 (81)/17 (19)
Number of prior regimens, 1/2/3	63 (69)/26 (29)/2 (2)
Number of prier platinum agent courses	4 (1 to 30)
Metastatic sites, liver/lung/bone/lymph node	17(19)/24(27)/ 15(17)/68(75)
Number of metastatic organs, 1/2/3/4/5	22(24)/40(44)/19(21)/ 6(7)/4(4)
WBC (/ml)*	5,500 (1,800 to 20,270)
Hb (mg/dl)*	10.4 (6.6 to 16.1)
Platelet count (/ml)*	231,000 (60,000 to 713,000)
Neutrophil count (/ml)*	3,674 (734 to 17,757)
Lymphocyte count (/ml)*	1,179 (385 to 2,604)
Monocyte count (/ml)*	420 (15 to 1245)
NLR*	2.80 (0.75 to 20.25)
MLR*	0.35 (0.01 to 1.60)
SIRI*	1.28 (0.02 to 13.00)
PLR*	181.5 (54.9 to 768.7)
WBC (/ml) at 1 month	6,100 (1,730 to 27,800)
Hb (mg/dl) at 1 month	10.5 (6.1 to 16.3)
Platelet count (/ml) at 1 month	205,000 (27,000 to 543,000)
Neutrophil count (/ml) at 1 month	4,176 (190 to 25,993)
Lymphocyte count (/ml) at 1 month	1,123 (190 to 2,538)
Monocyte count (/ml) at 1 month	402 (10 to 1710)
NLR at 1 month	3.91 (0.20 to 95.00)
MLR at 1 month	0.35 (0.01 to 4.00)
SIRI at 1 month	1.35 (0.03 to 80.94)
PLR at 1 month	168.1 (21.06 to 824.72)

UC: Urinary carcinoma; EORTG PS: Eastern Cooperative Oncology Group Performance status; WBC: white blood cell count; Hb: hemoglobin; NLR: neutrophil-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; SIRI: systemic inflammation response index; PLR: platelet-lymphocyte ratio. *preinitiation data.

Results

The present study included 91 advanced UC patients who were treated with pembrolizumab as second-line treatment, after failure of platinum-based chemotherapy, at Kanazawa University or one of seven related hospitals. The characteristics of the patients are presented in Table I. The median change from pretreatment to 1 month, or the 1-month change, was +39.6% for the NLR, 0% for the MLR, +5.5% for the SIRI, and –9.7% for the PLR. The median observation period for the patients was 7.9 months (range=0.4-24.8 months), and 51 (57%) patients died within the observation

Table II. *Correlation coefficient between each blood cell counts.*

		Neutrophil*	Lymphocyte*	Monocyte	Platelet-change	Neutrophil-change	Lymphocyte-change	Monocyte-change
Platelet count*	CC	0.426	–0.007	0.283	0.687	0.321	–0.281	–0.209
	p-value	<0.001	0.945	0.007	<0.001	0.002	0.007	0.047
Neutrophil count*	CC		0.056	0.43	0.373	0.562	–0.511	–0.325
	p-value		0.599	<0.001	<0.001	<0.001	<0.001	0.002
Lymphocyte count*	CC			0.141	0.016	–0.436	0.521	0.196
	p-value			0.181	0.882	<0.001	0.015	0.620
Monocyte count*	CC				0.213	0.146	–0.202	0.222
	p-value				0.043	0.168	0.055	0.035
Platelet-change	CC					0.241	–0.208	–0.124
	p-value					0.021	0.048	0.243
Neutrophil-change	CC						–0.904	–0.524
	p-value						<0.001	<0.001
Lymphocyte-change	CC							0.416
	p-value							<0.001

CC: Correlation coefficient. *preinitiation data.

Table III. *Correlation coefficient between each blood cell count parameters.*

		WBC*	Hb*	Plt*	MLR*	SIRI*	PLR*	NLR-change	MLR-change	SIRI-change	PLR-change
NLR*	CC	0.590	–0.341	0.349	0.617	0.801	0.625	–0.053	–0.152	–0.097	–0.075
	p-Value	<0.001	0.001	0.001	<0.001	<0.001	<0.001	0.620	0.150	0.360	0.477
MLR*	CC	0.229	–0.277	0.189		0.853	0.522	0.060	–0.450	–0.236	–0.118
	p-Value	0.029	0.008	0.073		<0.001	<0.001	0.573	<0.001	0.024	0.265
SIRI*	CC	0.636	–0.227	0.359			0.488	–0.036	–0.362	–0.255	–0.053
	p-Value	<0.001	0.030	<0.001			<0.001	0.736	<0.001	0.015	0.620
PLR*	CC	0.116	–0.398	0.708				0.131	–0.201	–0.043	–0.400
	p-Value	0.275	<0.001	<0.001				0.216	0.056	0.683	<0.001

CC: Correlation coefficient; WBC: white blood cell count (/ml); Hb: hemoglobin (mg/dl); Plt: platelet count (/ml); NLR: neutrophil-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; SIRI: systemic inflammation response index; PLR: platelet-lymphocyte ratio. *preinitiation data. Each change calculated as % change such as [(NLR at 1 month/pretreatment NLR) - 1] ×100.

period. The median time-to-death for these cases was 3.7 months. The median observation period for surviving patients was 15.4 months (range=2.9-24.8 months). Thirty-four patients (37.4%) died within 6 months after starting pembrolizumab. Kaplan–Meier analysis indicated that the median OS for the patients was 11.8 months (95%CI=5.3-18.4). The best responses to treatment were shown in 9 cases (9.9%) of complete response (CR), 15 cases (16.5%) of partial response (PR), 25 cases (27.5%) of stable disease, and 42 cases (46.1%) of progressive disease (PD). Pretreatment platelet, neutrophil and lymphocyte counts were positively correlated with changes at 1-month post-treatment (CCs: 0.687, 0.562, and 0.521, respectively, all $p<0.001$). Percent changes of neutrophil count at one month after treatment were strongly inversely correlated with percent changes of lymphocyte count at one month (CC: −0.904, $p<0.001$) (Table II). Pretreatment NLR was strongly correlated with pretreatment MLR, SIRI, and PLR (CCs: 0.617, 0.801, and 0.625, respectively, all $p<0.001$) (Table III). Cox regression analysis was performed for each pretreatment factor and for the rate of change in relation to OS after pembrolizumab initiation; the univariate analysis indicated that OS was significantly associated with pretreatment NLR, MLR, SIRI, and PLR, as well as the 1-month-change NLR (Table IV). Among the pretreatment NLR, MLR, SIRI, and PLR, which were predictive factors for OS after pembrolizumab initiation in univariate analysis, pretreatment NLR was further examined by multivariate analysis, because both pretreatment NLR and MLR had the lowest p-values among NLR, MLR, SIRI, and PLR, and the p-value of pretreatment neutrophil

Table IV. *Univariate and multivariate analyses of predictive factors for overall survival with pembrolizumab therapy*

	Univariate		Multivariate	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
WBC (/ml)*	1.000 (1.000-1.000)	0.348		
Hb (mg/dl)*	0.774 (0.668-0.896)	0.001	0.882 (0.757-1.027)	0.107
Platelet count (/ml)*	1.000 (1.000-1.000)	0.089		
Neutrophil count (/ml)*	1.000 (1.000-1.000)	0.109		
Lymphocyte count (/ml)*	0.941 (0.911-0.972)	<0.001		
Monocyte count (/ml)*	1.000 (0.999-1.002)	0.405		
NLR*	1.190 (1.106-1.280)	<0.001	1.180 (1.089-1.279)	<0.001
MLR*	1.178 (1.094-1.268)	<0.001		
SIRI*	2.786 (1.424-5.451)	0.003		
PLR*	1.002 (1.001-1.004)	0.001		
1-month change Hb	0.971 (0.942-1.001)	0.062		
1-month change platelet	0.995 (0.987-1.004)	0.286		
1-month change neutrophil	1.015 (1.002-1.028)	0.023		
1-month change lymphocyte	0.984 (0.974-0.994)	0.001		
1-month change monocyte	1.000 (0.998-1.001)	0.55		
1-month change NLR	1.004 (1.002-1.005)	<0.001	1.003 (1.002-1.005)	<0.001
1-month change MLR	1.000 (0.999-1.001)	0.699		
1-month change SIRI	1.001 (1.000-1.001)	0.033		
1-month change PLR	0.999 (0.993-1.004)	0.624		

HR: Hazard ratio; CI: confidence interval; WBC: white blood cell count; Hb: Hemoglobin; NLR: neutrophil-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; SIRI: systemic inflammation response index; PLR: platelet-lymphocyte ratio. *preinitiation data. Each change calculated as % change such as [(NLR at 1 month/pretreatment NLR) - 1] ×100.

count was lower than that of pretreatment monocyte count (Table IV). In the multivariate analysis, the pretreatment NLR and the 1-month-change NLR all showed a significant effect on OS after pembrolizumab initiation (Table IV). From the results of the HR and *p*-value at each cutoff point calculated using Cox proportional hazard model, the cutoff points for pretreatment NLR and 1-month change NLR were 2.9, +43%, respectively (Figure 1), and we compared the OS rates for the four groups at those cutoffs. The pretreatment NLR <2.9 and 1-month-change NLR <43% groups had a significantly better prognosis than the pretreatment NLR ≥2.9 and 1-month-change NLR ≥43% groups (Figure 2).

Discussion

In this study, we investigated peripheral blood cell count markers, including the pretreatment NLR, PLR, SIRI, and MLR, before treatment with pembrolizumab and the post-treatment changes in them in relation to prognosis. There have been reports on the pre-ICI NLR and its changes at post-treatment, as well as on the pretreatment PLR. The relationship between prognosis and the pre-ICI treatment NLR and its changes at post-treatment, as well as the relationship between pre-ICI treatment PLR and prognosis has been reported (7-9, 11, 12). To the best of our knowledge, this is the first report of an analysis of the relationship between prognosis and SIRI and MLR before ICI, as well as the relationship between prognosis and changes in PLR, SIRI, MLR, and each peripheral blood cell count after ICI. In the present study, the univariate analysis of factors for OS with pembrolizumab treatment showed that low Hb, low lymphocyte count, high NLR, high MLR, high SIRI, and high PLR at pretreatment and neutrophil, NLR and SIRI increase, lymphocyte decrease at 1-month post-treatment were significant predictors of poor prognosis (Table IV). With regard to pretreatment markers, the denominators of NLR, MLR, SIRI, and PLR were all lymphocyte counts, and univariate analysis showed that low lymphocyte counts before pembrolizumab initiation were a poor prognostic factor for OS (Table IV), suggesting that lymphocyte counts had a significant effect on the cause of the poor prognosis of elevated NLR, MLR, SIRI, and PLR. It has been reported that high pretreatment NLR levels are a predictor of poor prognosis after ICI treatment for several types of carcinomas, including UC (7, 12). Similarly, it has been reported that high pretreatment PLR is a predictor of poor prognosis in some carcinomas (11). There have been reports of patients with an elevated NLR having a poor prognosis after undergoing ICI treatment (9). However, Li et al. reported from their investigation of 509 patients treated with ICI that patients who had a moderate decrease in the NLR during ICI treatment were found to have the longest survival, whereas a significant

Figure 1. *Plotted lines of the hazard ratio (HR) and p-value at each cutoff of points of preinitiation neutrophil–lymphocyte ratio (NLR) (A) and 1-month change NLR (B) calculated using Cox proportional hazard model for predicting overall survival.*

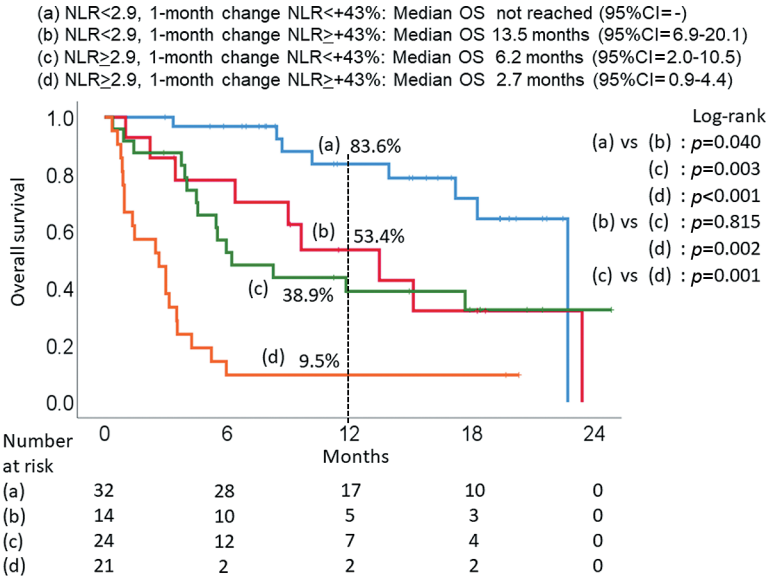

Figure 2. *Kaplan–Meier curves of overall survival (OS) based on the pretreatment neutrophil–lymphocyte ratio (NLR) and 1-month-change NLR for each patient.*

decrease or increase in the NLR was associated with a shorter survival (8). In the present study, overall, an elevated NLR was a predictor of a poor prognosis. However, in a small number of cases of death within six months, there was a large decrease in the NLR. Studies with a larger sample size should be performed in the future. The pretreatment NLR, MLR, SIRI, and PLR were each relatively strongly correlated with OS (Table III). Among pretreatment NLR, MLR, SIRI, and PLR, we selected pretreatment NLR for multivariate analysis of OS, because of the high HR and p-value of this variable in univariate analysis (Table IV). In the multivariate analysis selecting the pretreatment Hb, NLR, and 1-month-change NLR, which were significant in the univariate analysis of OS, the pretreatment NLR and 1-month-change NLR were significant predictors (Table IV). In the present study, the reason why the NLR change after treatment was useful as a predictive marker was that both the increase in neutrophils and the decrease in lymphocytes after the initiation of

pembrolizumab were poor prognostic factors (Table IV), and the number of neutrophils and lymphocytes after the initiation of pembrolizumab showed a strong inverse correlation (Table II). When the current cohort was grouped according to the cutoff percentages of these two factors, and OS was compared by the Kaplan–Meier method, the group with a low pretreatment NLR and low 1-month-change NLR elevation had a 1-year survival of 83.6%, while the group with a high pretreatment NLR and 1-month-change NLR elevation had a higher rate of death within six months (Figure 2). These results suggest that even a simple index such as the NLR can be used to predict the effect of ICI treatment to some extent.

Many aspects of the immune response in tumors induced by ICI remain unclear. The reasons why the NLR, MLR, SIRI, and PLR, were found to be significant predictors of prognosis in the present study are as follows. Tumors induce inflammatory reactions through the activation of transcription factors, such as nuclear factor-κB (NF-κB) (13). Tumor-derived cytokines and growth factors are secreted into the systemic circulation to mediate communication with distant sites. Therefore, inflammatory responses caused by cancer may affect the numbers of blood cell components, such as systemic neutrophils, lymphocytes, monocytes, and platelets (14-16). Lymphocytes play an important role in the immune system, and their decrease leads to immunological disorders. It has been reported that high levels of CD4+ T lymphocytes at the tumor margin inhibit tumor progression, whereas a decrease in the number of lymphocyte subsets (such as CD4+, CD8+, CD3+, and CD56+ T cells) attenuates the antitumor cellular immune response (17). In addition, circulating lymphocytes secrete cytokines, which inhibit tumor cell proliferation and metastasis and can have a cytotoxic effect on cancer cells (18). Neutrophils play important roles in different aspects of cancer development and progression, specifically in tumor angiogenesis and metastasis, through cytokines such as vascular endothelial growth factor, tumor necrosis factor-α (TNF-α), and tumor growth factor-β (TGF-β) (19). Neutrophils also affect immunomodulation. Therefore, suppressing the activity of T cell responses may promote tumor progression and metastasis (20). Responses to ICI cause global changes in the composition of the tumor microenvironment. Increased numbers of CD8+ T cells and NK cells were observed in the tumors of patients who responded to ICI (21). Tumors responding to ICI also revealed an increased number of B cells and increased formation of tertiary lymphoid structures (22, 23). T cell receptor repertoire clonality, which is the expansion of specific T cell clones, was associated with a response to ICI (21, 24). This clonality was also observed in peripheral T cells (25). These findings are evidence that a localized response to ICI can be observed in the peripheral blood, and the changes observed in the present study may reflect this localized response in tumors.

There are several limitations to our study. This was a retrospective study. Therefore, unknown sources of bias, including a heterogenous patient population, and variables that could not be evaluated, may exist. The decision to introduce pembrolizumab was up to the physician in charge, and there was some variation among physicians in the timing when the blood draw was performed. There is no clear explanation of how peripheral blood and its fractions, from which the NLR, MLR, SIRI, and PLR calculations are derived, how they relate to inflammation in the tumor tissue and the specific mechanisms involved. Also, the effects that ICI has on the inflammatory response in tumor tissue and prognosis are not yet fully understood. Further studies, including prospective ones, with a larger patient cohort should be performed in the future.

In conclusion, NLR, MLR, PLR and SIRI before pembrolizumab and 1-month-change NLR in advanced UC correlated with OS after pembrolizumab treatment especially affected by lymphocyte counts. One month after pembrolizumab initiation, the increased lymphocyte count and decreased neutrophil count were found to be useful in predicting a better prognosis from pembrolizumab treatment, and the 1-month change in neutrophils and lymphocytes after the initiation of pembrolizumab showed a strong inverse correlation.

Conflicts of Interest

The Authors have stated that they have no conflicts of interest in relation to this study.

Authors' Contributions

Yoshifumi Kadono: conceptualization, methodology, writing and original draft preparation. Shohei Kawaguchi: data curation, writing and original draft preparation. Takahiro Nohara: visualization and investigation. Kazuyoshi Shigehara: data curation and investigation. Kouji Izumi: conceptualization and methodology. Taiki Kamijima: investigation and validation. Chikashi Seto: supervision, reviewing and editing. Akinobu Takano: investigation and data curation. Satoshi Yotsuyanagi: data curation and supervision. Ryunosuke Nakagawa: investigation and validation. Tohru Miyagi: supervision and formal analysis. Shuhei Aoyama: investigation and visualization. Hideki Asahi: investigation and formal analysis. Rie Fukuda: investigation and validation. Atsushi Mizokami: reviewing, editing and project administration. All Authors read and approved the final manuscript.

Acknowledgements

The Authors would like to thank the following individuals and institutions participated in this study for their invaluable help with data collection: Hiroaki Iwamoto, Hiroshi Yaegashi, Masashi Iijima of the Department of Integrative Cancer Therapy and Urology in Kanazawa University Graduate School of Medical Science Kanazawa, Kanazawa, Japan; Yuuta Takezawa, Kazuaki Machioka of the Department of Urology, Toyama Prefectural Central Hospital, Toyama, Japan; Jirou

Sakamoto of the Department of Urology, Kouseiren Takaoka Hospital, Takaoka, Toyama, Japan; Kazuhiko Shibata of the Department of Medical Oncology, Kouseiren Takaoka Hospital, Takaoka, Toyama, Japan; Satoko Urata, Mitsuo Ofude, Takao Nakashima of the Department of Urology, Ishikawa Prefectural Central Hospital, Kanazawa, Ishikawa, Japan; Hiderou Minami, Osamu Ueki of the Department of Urology, Noto General Hospital, Nanao, Ishikawa, Japan; Masaharu Nakai, Kazunori Kobashi of the Department of Urology, Kaga Medical Center, Kaga, Ishikawa, Japan; Masato Yagisawa, Masashi Takeda, Hidekazu Yamamoto of the Department of Urology, Fukui-ken Saiseikai Hospital, Fukui, Japan; Atsuya Takimoto, Ryou Satou, Sotaro Miwa, Kiyoshi Koshida of the National Hospital Organization Kanazawa Medical Center, Kanazawa, Japan.

References

1 Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ, Climent MA, Petrylak DP, Choueiri TK, Necchi A, Gerritsen W, Gurney H, Quinn DI, Culine S, Sternberg CN, Mai Y, Poehlein CH, Perini RF, Bajorin DF and KEYNOTE-045 Investigators: Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 376(11): 1015-1026, 2017. PMID: 28212060. DOI: 10.1056/NEJMoa1613683

2 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144(5)*: 646-674, 2011. PMID: 21376230. DOI: 10.1016/j.cell.2011.02.013

3 Pichler M, Hutterer GC, Stoeckigt C, Chromecki TF, Stojakovic T, Golbeck S, Eberhard K, Gerger A, Mannweiler S, Pummer K and Zigeuner R: Validation of the pre-treatment neutrophil-lymphocyte ratio as a prognostic factor in a large European cohort of renal cell carcinoma patients. Br J Cancer *108(4)*: 901-907, 2013. PMID: 23385728. DOI: 10.1038/bjc.2013.28

4 Dalpiaz O, Krieger D, Ehrlich GC, Pohlmann K, Stojakovic T, Pummer K, Zigeuner R, Pichler M and Hutterer GC: Validation of the preoperative platelet-to-lymphocyte ratio as a prognostic factor in a european cohort of patients with upper tract urothelial carcinoma. Urol Int *98(3)*: 320-327, 2017. PMID: 27732981. DOI: 10.1159/000452109

5 Chen Z, Wang K, Lu H, Xue D, Fan M, Zhuang Q, Yin S, He X and Xu R: Systemic inflammation response index predicts prognosis in patients with clear cell renal cell carcinoma: a propensity score-matched analysis. Cancer Manag Res *11*: 909-919, 2019. PMID: 30697081. DOI: 10.2147/CMAR.S186976

6 Jan HC, Yang WH and Ou CH: Combination of the preoperative systemic immune-inflammation index and monocyte-lymphocyte ratio as a novel prognostic factor in patients with upper-tract urothelial carcinoma. Ann Surg Oncol *26(2)*: 669-684, 2019. PMID: 30374917. DOI: 10.1245/s10434-018-6942-3

7 Sacdalan DB, Lucero JA and Sacdalan DL: Prognostic utility of baseline neutrophil-to-lymphocyte ratio in patients receiving immune checkpoint inhibitors: a review and meta-analysis. Onco Targets Ther *11*: 955-965, 2018. PMID: 29503570. DOI: 10.2147/OTT.S153290

8 Li M, Spakowicz D, Burkart J, Patel S, Husain M, He K, Bertino EM, Shields PG, Carbone DP, Verschraegen CF, Presley CJ, Otterson GA, Kendra K and Owen DH: Change in neutrophil to lymphocyte ratio during immunotherapy treatment is a non-linear predictor of patient outcomes in advanced cancers. J Cancer Res Clin Oncol *145(10)*: 2541-2546, 2019. PMID: 31367835. DOI: 10.1007/s00432-019-02982-4

9 Ota Y, Takahari D, Suzuki T, Osumi H, Nakayama I, Oki A, Wakatsuki T, Ichimura T, Ogura M, Shinozaki E, Suenaga M, Chin K and Yamaguchi K: Changes in the neutrophil-to-lymphocyte ratio during nivolumab monotherapy are associated with gastric cancer survival. Cancer Chemother Pharmacol *85(2)*: 265-272, 2020. PMID: 31907646. DOI: 10.1007/s00280-019-04023-w

10 Cohen J: The effect size. In: Statistical Power Analysis for the Behavior Science. Cohen J (ed.). 2nd edn. Lawrence Erlbaum Associates, Hillsdale, NJ, pp. 77-83, 1988.

11 Xu H, He A, Liu A, Tong W and Cao D: Evaluation of the prognostic role of platelet-lymphocyte ratio in cancer patients treated with immune checkpoint inhibitors: A systematic review and meta-analysis. Int Immunopharmacol *77*: 105957, 2019. PMID: 31677498. DOI: 10.1016/j.intimp.2019.105957

12 Ogihara K, Kikuchi E, Shigeta K, Okabe T, Hattori S, Yamashita R, Yoshimine S, Shirotake S, Nakazawa R, Matsumoto K, Mizuno R, Hara S, Oyama M, Masuda T, Niwakawa M and Oya M: The pretreatment neutrophil-to-lymphocyte ratio is a novel biomarker for predicting clinical responses to pembrolizumab in platinum-resistant metastatic urothelial carcinoma patients. Urol Oncol *38(6)*: 602.e1-602.e10, 2020. PMID: 32139290. DOI: 10.1016/j.urolonc.2020.02.005

13 Tsujimoto H, Ono S, Ichikura T, Matsumoto Y, Yamamoto J and Hase K: Roles of inflammatory cytokines in the progression of gastric cancer: friends or foes? Gastric Cancer *13(4)*: 212-221, 2010. PMID: 21128056. DOI: 10.1007/s10120-010-0568-x

14 Grivennikov SI, Greten FR and Karin M: Immunity, inflammation, and cancer. Cell *140(6)*: 883-899, 2010. PMID: 20303878. DOI: 10.1016/j.cell.2010.01.025

15 McMillan DC: The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer. Cancer Treat Rev *39(5)*: 534-540, 2013. PMID: 22995477. DOI: 10.1016/j.ctrv.2012.08.003

16 Diakos CI, Charles KA, McMillan DC and Clarke SJ: Cancer-related inflammation and treatment effectiveness. Lancet Oncol *15(11)*: e493-503, 2014. PMID: 25281468. DOI: 10.1016/S1470-2045(14)70263-3

17 Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH and Pagès F: Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science *313(5795)*: 1960-1964, 2006. PMID: 17008531. DOI: 10.1126/science.1129139

18 Ding PR, An X, Zhang RX, Fang YJ, Li LR, Chen G, Wu XJ, Lu ZH, Lin JZ, Kong LH, Wan DS and Pan ZZ: Elevated preoperative neutrophil to lymphocyte ratio predicts risk of recurrence following curative resection for stage IIA colon cancer. Int J Colorectal Dis *25(12)*: 1427-1433, 2010. PMID: 20821217. DOI: 10.1007/s00384-010-1052-0

19 Liang W and Ferrara N: The complex role of neutrophils in tumor angiogenesis and metastasis. Cancer Immunol Res *4(2)*: 83-91, 2016. PMID: 26839309. DOI: 10.1158/2326-6066.CIR-15-0313

20 De Larco JE, Wuertz BR and Furcht LT: The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. Clin Cancer Res *10(15)*: 4895-4900, 2004. PMID: 15297389. DOI: 10.1158/1078-0432.CCR-03-0760

21 Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, Hodi FS, Martín-Algarra S, Mandal R, Sharfman WH, Bhatia S,

Hwu WJ, Gajewski TF, Slingluff CL Jr, Chowell D, Kendall SM, Chang H, Shah R, Kuo F, Morris LGT, Sidhom JW, Schneck JP, Horak CE, Weinhold N and Chan TA: Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell *171(4)*: 934-949.e16, 2017. PMID: 29033130. DOI: 10.1016/j.cell.2017.09.028

22 Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, Johansson I, Phung B, Harbst K, Vallon-Christersson J, van Schoiack A, Lövgren K, Warren S, Jirström K, Olsson H, Pietras K, Ingvar C, Isaksson K, Schadendorf D, Schmidt H, Bastholt L, Carneiro A, Wargo JA, Svane IM and Jönsson G: Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature *577(7791)*: 561-565, 2020. PMID: 31942071. DOI: 10.1038/s41586-019-1914-8

23 Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, Yizhak K, Sade-Feldman M, Blando J, Han G, Gopalakrishnan V, Xi Y, Zhao H, Amaria RN, Tawbi HA, Cogdill AP, Liu W, LeBleu VS, Kugeratski FG, Patel S, Davies MA, Hwu P, Lee JE, Gershenwald JE, Lucci A, Arora R, Woodman S, Keung EZ, Gaudreau PO, Reuben A, Spencer CN, Burton EM, Haydu LE, Lazar AJ, Zapassodi R, Hudgens CW, Ledesma DA, Ong S, Bailey M, Warren S, Rao D, Krijgsman O, Rozeman EA, Peeper D, Blank CU, Schumacher TN, Butterfield LH, Zelazowska MA, McBride KM, Kalluri R, Allison J, Petitprez F, Fridman WH, Sautès-Fridman C, Hacohen N, Rezvani K, Sharma P, Tetzlaff MT, Wang L and Wargo JA: B cells and tertiary lymphoid structures promote immunotherapy response. Nature *577(7791)*: 549-555, 2020. PMID: 31942075. DOI: 10.1038/s41586-019-1922-8

24 Inoue H, Park JH, Kiyotani K, Zewde M, Miyashita A, Jinnin M, Kiniwa Y, Okuyama R, Tanaka R, Fujisawa Y, Kato H, Morita A, Asai J, Katoh N, Yokota K, Akiyama M, Ihn H, Fukushima S and Nakamura Y: Intratumoral expression levels of *PD-L1*, *GZMA*, and *HLA-A* along with oligoclonal T cell expansion associate with response to nivolumab in metastatic melanoma. Oncoimmunology *5(9)*: e1204507, 2016. PMID: 27757299. DOI: 10.1080/2162402X.2016.1204507

25 Wu TD, Madireddi S, de Almeida PE, Banchereau R, Chen YJ, Chitre AS, Chiang EY, Iftikhar H, O'Gorman WE, Au-Yeung A, Takahashi C, Goldstein LD, Poon C, Keerthivasan S, de Almeida Nagata DE, Du X, Lee HM, Banta KL, Mariathasan S, Das Thakur M, Huseni MA, Ballinger M, Estay I, Caplazi P, Modrusan Z, Delamarre L, Mellman I, Bourgon R and Grogan JL: Peripheral T cell expansion predicts tumour infiltration and clinical response. Nature *579(7798)*: 274-278, 2020. PMID: 32103181. DOI: 10.1038/s41586-020-2056-8

Received January 31, 2021
Revised February 13, 2021
Accepted February 14, 2021