ORIGINAL ARTICLE

Association between peripheral eosinophils and clinical outcomes in patients with non–small cell lung cancer treated with immune checkpoint inhibitors

Shinichiro Okauchi[1], Toshihiro Shiozawa[2], Kunihiko Miyazaki[3], Kengo Nishino[1], Yuika Sasatani[1], Gen Ohara[1], Katsunori Kagohashi[1], Shinya Sato[2], Takahide Kodama[2], Hiroaki Satoh[1], Nobuyuki Hizawa[2]

1 Division of Respiratory Medicine, Mito Medical Center, University of Tsukuba–Mito Kyodo General Hospital, Mito, Japan
2 Division of Respiratory Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan
3 Division of Respiratory Medicine, Ryugasaki Saiseikai Hospital, Ryugasaki, Japan

KEY WORDS

immune checkpoint inhibitor, non–small cell lung cancer, peripheral eosinophils, predictive factor, survival

ABSTRACT

INTRODUCTION Programmed cell death ligand 1 is considered a predictor of the therapeutic effect of immune checkpoint inhibitors (ICPIs), but a more simple and useful predictor is needed.

OBJECTIVES The aim of this study was to identify the relationship between eosinophil counts and percentages and response to ICPI therapy.

PATIENTS AND METHODS In 190 patients with non–small cell lung cancer (NSCLC) treated with ICPI therapy, peripheral eosinophil counts and percentages at the time of ICPI therapy initiation, the maximum counts and percentages of eosinophils during ICPI therapy, response to therapy, and time to treatment failure (TTF) were investigated.

RESULTS Both an increase in the peripheral eosinophil count and an elevation of eosinophil percentage following the initiation of ICPI therapy were observed, regardless of whether the patients had controlled or progressive disease. The median time to the maximum eosinophil percentage was 5 weeks in patients with controlled disease and 2 weeks in those with progressive disease. The cutoff value for the maximum eosinophil counts and percentage during ICPI therapy was set at 300/μl and 5%, respectively, to identify the presence or absence of a therapeutic effect. Time to treatment failure was longer in patients with maximum eosinophil counts exceeding 300/μl and a maximum eosinophil percentage above 5%. In a multivariable analysis, a maximum eosinophil percentage of 5% during ICPI therapy was a significant predictive factor for therapeutic efficacy.

CONCLUSIONS The measurement of peripheral eosinophils up to around 5 weeks following the initiation of treatment, especially the maximum eosinophils count and percentage, might provide useful information about the efficacy of ICPIs.

Correspondence to:
Hiroaki Satoh, MD, PhD, Division of Respiratory Medicine, Mito Medical Center, University of Tsukuba–Mito Kyodo General Hospital, 3-2-7, Miya-machi, Mito, Ibaraki, 310-0105, Japan, phone: +81 29 231 2371, email: hirosato@md.tsukuba.ac.jp
Received: October 21, 2020.
Revision accepted: December 27, 2020.
Published online: January 25, 2021.
Pol Arch Intern Med. 2021; 131 (2): 152-160
doi:10.20452/pamw.15776

INTRODUCTION Immune checkpoint inhibitors (ICPIs) have dramatically changed the treatment of non–small cell lung cancer (NSCLC) in recent years by demonstrating an overall survival benefit.[1] At present, ICPIs are being used as the first-line treatment with or without chemotherapy. Programmed cell death ligand 1 (PD-L1) is the only established biomarker for predicting the efficacy of ICPI therapy.[1] Currently, PD-L1 expression is used as a companion diagnostic parameter to clinical markers and a predictive indicator of the therapeutic efficacy to anti-PD-1/PD-L1 antibodies.[2] It is well known that using the immunostaining method for PD-L1 expression evaluation has several limitations.[3] Namely, it does not show how the expression changes over time, eg, in

WHAT'S NEW?

Immune checkpoint inhibitors (ICPIs) have revolutionized the treatment of patients with various types of carcinoma. The effect of this therapy on long-term survival is epoch making, but the benefits are limited to a certain group of patients. Also, it is difficult to identify patients who would benefit from the therapy before initiating treatment. Programmed cell death ligand 1 expression has been used as a biomarker for ICPI therapy. However, it exhibits a nonuniform immunostaining pattern across the cancer tissue, which leads to inaccuracies in interpreting the overall expression. In this study, we identified eosinophil percentages greater than 5% during ICPI therapy as a significant factor for the prediction of ICPI therapeutic efficacy in a multivariable analysis. It would not be difficult or expensive to include the measurement of eosinophils when measuring complete blood count and to observe the association of eosinophil counts and percentages with the efficacy of ICPI therapy. It may be possible to predict patient response to ICPI therapy based on peripheral eosinophils.

response to interferon γ induction in the tumor microenvironment.[3] Therefore, even in the same patient, the expression of PD-L1 may differ depending on the time of sample collection.[3] In addition, PD-L1 expression in tumors is not uniform and it tends to be localized around interferon γ–producing T cells, resulting in differences between measurements at various biopsy sites.[3] Considering the challenges related to the methodology and interpretation of PD-L1 immunostaining, other predictive factors have been sought. In recent years, it has been reported that peripheral blood leukocytes may be a promising predictor in ICPI therapy.[4-19] A study by Tanizaki et al[11] demonstrated that a low absolute neutrophil count, high absolute lymphocyte count, and high absolute eosinophil count were significantly and independently associated with better survival. In that study, blood samples were obtained only within 7 days before ICPI therapy initiation. We also encountered a patient with a high peripheral blood eosinophil count and a successful response to ICPI therapy.[20] In clinical practice, determination of complete blood count (CBC) is essential for chemotherapy of advanced lung cancer. If investigating the number of eosinophils and their percentage could help us predict the efficacy of ICPI therapy, it would be a cheap and convenient indicator. Eosinophils are known to play an important role in parasitic and allergic diseases, and the number of peripheral blood eosinophils is known to be increased in those patients.[21] On the other hand, eosinophil infiltration into tumor lesions and peripheral eosinophilia have been reported in patients with cancer.[22-25] The exact mechanism and clinical importance of eosinophilia in peripheral blood and tumor tissue remain unclear. However, attempts have been made to elucidate it, albeit little by little, eg, the involvement of cytokines such as interleukin 5 and interleukin 16 was examined.[10,24] As mentioned above, ICPIs have become common antitumor drugs in the treatment of cancer in various organs, and more attention is being paid to the relationship between eosinophils and cancer immunity.[10,17,26,27] The role of eosinophils in cancer immunity remains unknown; however, the relationship among peripheral eosinophils and antitumor effects, response duration, and survival has recently been of interest.[6-8,10,11,16-18,26] As currently there is no valid biomarker to replace PD-L1, expectations associated with the role of peripheral eosinophils as biomarkers for ICPI efficacy have been rising.[6-8,10,11,16-18,26] Yet, there are some unclear issues pertaining to the significance of peripheral blood eosinophils in ICPI therapy. We do not know what is of greater prognostic value: the eosinophil count before ICPI therapy initiation[11,12] or a change in the eosinophil count after starting treatment.[6,8,10,15,17,18] Even if changes in the eosinophil count after ICPI therapy initiation are more relevant, it is unclear when the measured eosinophil count is meaningful. Furthermore, there has been uncertainty as to whether the relative increase in eosinophils or an increase in absolute numbers is crucial.

In the present study, we focused on peripheral eosinophils and aimed to clarify the relationship between eosinophil counts and percentages and the efficacy of ICPI therapy. This study was performed to determine whether eosinophils before or during ICPI therapy had a greater impact on patient response and whether eosinophil counts or percentages were a better predictor of therapeutic response.

PATIENTS AND METHODS

Patients We analyzed the medical records of all patients diagnosed with NSCLC in 3 tertiary hospitals in Japan (Mito Medical Center, University of Tsukuba–Mito Kyodo General Hospital, Ryugasaki Saiseikai Hospital, and Tsukuba University Hospital) between February 2016 and December 2019. Patients with NSCLC treated with ICPI monotherapy, or combination therapy with ICPI and chemotherapy, during this period were included in this study. Lung cancer was diagnosed based on the World Health Organization classification. Tumor-node-metastasis staging (TNM Classification, 8th edition) using head computed tomography or magnetic resonance imaging, bone scans, and ultrasonography and/or computed tomography of the abdomen was performed in all patients prior to ICPI therapy initiation. Patients with the following comorbidities and with a history of treatment for these conditions were excluded: parasitic infestations, allergic diseases, autoimmune diseases, and hematologic malignancies. Patients with chronic obstructive pulmonary disease and those with bronchial asthma and chronic obstructive pulmonary disease overlap requiring systemic steroid use were also excluded. Particular attention was paid to adrenal insufficiency as an immune-related adverse event (irAE). Patients who developed eosinophilia associated with adrenal insufficiency as an irAE were excluded from this study. Patient demographic data including age, sex, Eastern Cooperative Oncology

TABLE 1 Characteristics of 190 study patients with non–small cell lung cancer treated with immune checkpoint inhibitor therapy

Characteristic		ICPI monotherapy (n = 157)	ICPI + CBDCA-containing chemotherapy (n = 33)	*P* value
Age, y, median (IQR)		69 (63–76)	69 (64–74)	0.55
Sex	Male	124	25	0.65
	Female	33	8	
PS	0–1	133	30	0.43
	2–3	24	3	
Pathology	AD	95	18	0.47
	SQ	49	10	
	Other types[a]	13	5	
Stage	IIIA–C	46	7	0.39
	IVA	32	10	
	IVB	79	16	

Treatment regimens were as follows: 84 patients on nivolumab monotherapy, 77 on pembrolizumab (48 on pembrolizumab monotherapy, 15 on pembrolizumab + carboplatin + pemetrexed, and 14 on pembrolizumab + carboplatin + paclitaxel), and 29 on atezolizumab (25 on atezolizumab monotherapy, 2 on atezolizumab + carboplatin + pemetrexed, and 2 on atezolizumab + carboplatin + paclitaxel). Comparisons of means between the 2 independent groups were performed using the χ^2 or Mann–Whitney tests. Comparisons of 3 or more independent groups were performed using the Kruskal–Wallis test.

a Other types: 8 cases of poorly differentiated non–small cell lung cancer, 3 of large-cell neuroendocrine carcinoma, 3 of pleomorphic carcinoma, 2 of non–small cell lung cancer not otherwise specified, and 2 of adenosquamous cell carcinoma

Abbreviations: AD, adenocarcinoma; CBDCA, carboplatin; ICPI, immune checkpoint inhibitors; IQR, interquartile range; PS, performance status; SQ, squamous cell cancer

Group score for performance status, histopathology, disease stage, PD-L1 expression, objective tumor response, and survival were obtained from the patients' medical charts. Tumor response was evaluated as complete response, partial response, stable disease, or progressive disease, according to the Response Evaluation Criteria in Solid Tumors (Version 1.1).

Peripheral eosinophil count and percentage measurement Eosinophil counts and percentages were measured at the same time as CBC measurements before and during ICPI therapy. Results were obtained from the medical records of each patient. Counts for leukocyte subpopulations were measured by routine clinical laboratory analysis using the Sysmex XN-3000 analyzer (Sysmex Co., Ltd. Kobe, Japan).

Statistical analysis The χ^2 test was used to compare nominal variables. We used the nonparametric Mann–Whitney test to compare values with unknown population variance. Comparisons of 3 or more independent groups were performed using the Kruskal–Wallis test. To determine the cutoff value, a receiver operating characteristics (ROC) curve analysis was performed. The optimal cutoff was determined as the value for which the point on the ROC curve had the minimum distance to the upper left corner. Time to treatment failure (TTF) was used to evaluate prolonged therapeutic efficacy. We adopted the definition of TTF that is commonly used in cancer treatment: the interval from initiation of therapy with ICPIs to treatment discontinuation or the last follow-up visit. Time to treatment failure was estimated by the Kaplan–Meier method and compared using the log-rank test. We used the Cox proportional hazards model and forward-backward stepwise method to determine the independent variables used in the final model. Time to treatment failure was the dependent variable in that model. All statistical analyses were conducted using the BellCurve software for Excel, version 3.0 (SSRI Co, Ltd, Tokyo, Japan). A *P* value less than 0.05 was considered significant.

Ethics This study conformed to the Ethical Guidelines for Clinical Studies issued by the Ministry of Health, Labor, and Welfare of Japan. Written informed consent for a noninterventional retrospective study was obtained from each patient. The analysis of the medical records of patients with lung cancer was approved by the ethics committee of Mito Medical Center–University of Tsukuba Hospital (NO 16–66).

RESULTS

Patient characteristics We analyzed the clinical characteristics of 190 patients who met all inclusion criteria within the study period. Detailed data of the study patients are shown in TABLES 1 and 2. Of those 190 patients enrolled, 149 (78.4%) were male. The median age was 69 (range, 29–87) years. Lung cancers included 113 cases of adenocarcinoma, 59 of squamous cell carcinoma, and 18 of other types (8 cases of poorly differentiated NSCLC, 3 of large-cell neuroendocrine carcinoma, 3 of pleomorphic carcinoma, 2

TABLE 2 Characteristics of the study patients by the drugs used

Characteristic		ICPI monotherapy (n = 157)				ICPI + CBDCA-containing chemotherapy (n = 33)			*P* value
		P (n = 48)	N (n = 84)	A (n = 25)	PEM + A (n = 2)	PEM + P (n = 15)	Taxol + A (n = 2)	Taxol + P (n = 14)	
Age, y, median (IQR)		72 (68–78)	68 (60–76)	69 (60–73)	68 (59–76)	67 (65–72)	69 (64–75)	71 (65–76)	0.12
Sex	Male	41	66	17	2	10	2	11	0.5
	Female	7	18	8	0	5	0	3	
PS	0–1	38	71	24	2	13	2	13	0.52
	2–3	10	13	1	0	2	0	1	
Pathology	AD	30	46	19	2	13	2	1	0.29
	SQ	16	29	4	0	0	0	10	
	Other[a]	2	9	2	0	2	0	3	
Stage	IIIA-C	9	25	12	0	2	0	5	0.24
	IVA	14	18	0	0	7	1	2	
	IVB	25	41	13	2	6	1	7	

a Other types: 8 cases of poorly differentiated non–small cell lung cancer, 3 of large-cell neuroendocrine carcinoma, 3 of pleomorphic carcinoma, 2 of non–small cell lung cancer not otherwise specified, and 2 of adenosquamous cell carcinoma

Abbreviations: A, atezolizumab; N, nivolumab; P, pembrolizumab; PEM, pemetrexed; others, see **TABLE 1**

of NSCLC not otherwise specified, and 2 of adenosquamous cell carcinoma). A total of 136 patients had distant metastases. The ICPI monotherapy was started in 157 patients, and 33 patients received ICPI combined with carboplatin-containing chemotherapy. The following ICPIs were used: nivolumab in 84 patients, pembrolizumab in 77, and atezolizumab in 29. As shown in **TABLES 1** and **2**, there was no difference in the patients' baseline data in terms of treatment regimens used.

Distribution of peripheral eosinophils Peripheral eosinophil counts at the time of ICPI therapy initiation and the maximum eosinophil counts during ICPI therapy are presented as box plots in **FIGURE 1A** and **1B**, respectively. The distribution of the peripheral eosinophil percentage at the time of ICPI therapy initiation and the maximum eosinophil percentage during ICPI therapy are shown in **FIGURE 1C** and **1D**, respectively. When eosinophil counts increased in a patient, their eosinophil percentage increased as well.

Eosinophil measurement in response to immune checkpoint inhibitor therapy Eosinophil counts and percentages at the time of ICPI therapy initiation as well as the maximum eosinophil counts and percentages during ICPI therapy in the study patients who did or did not respond to ICPI therapy are shown in **TABLE 3**. There was a significant difference between patients with controlled disease and those with progressive disease in terms of eosinophil counts at the time of ICPI therapy initiation and the maximum eosinophil counts and percentages during ICPI therapy. In **TABLE 4**, we analyzed patients' data on peripheral eosinophils and response to ICPI therapy. Patients with complete response, partial response, stable disease, and progressive disease differed with regard to eosinophil counts at the time of ICPI therapy initiation and the maximum eosinophil counts and percentages during ICPI therapy. Changes in the percentage of eosinophils at the time of ICPI therapy initiation and the maximum percentage of eosinophils during ICPI therapy in each patient with NSCLC are shown in Supplementary material, *Figure S1*.

Determination of peripheral eosinophil cutoff values The ROC curve analysis was performed to determine the cutoff value that distinguished patients with controlled disease from those with progressive disease. The ROC curves for eosinophil counts and percentages at the time of ICPI therapy initiation and the maximum eosinophil counts and percentages during ICPI therapy are presented in **FIGURE 2**.

The area under the ROC curve for eosinophil counts and eosinophil percentages at the time of ICPI therapy initiation was 0.6106 (95% CI, 0.5198–0.7014) and 0.5849 (95% CI, 0.4934–0.6764), respectively. The area under the ROC curve for the maximum eosinophil counts and the maximum eosinophil percentage during the ICPI therapy was 0.6442 (95% CI, 0.5615–0.7269) and 0.6814 (95% CI, 0.6007–0.7621), respectively. The most appropriate cutoff values for patients with controlled disease and those with progressive disease were an eosinophil count of 330/μl and an eosinophil percentage of 5% during the ICPI therapy. When the cutoff value was set to 5%, the sensitivity of the model reached 60.7%, the specificity, 27.3%, and the odds ratio, 4.13.

Association of eosinophils at the time treatment failure with immune checkpoint inhibitors therapy Based on the cutoff values determined through the ROC curve analysis and those reported in previous

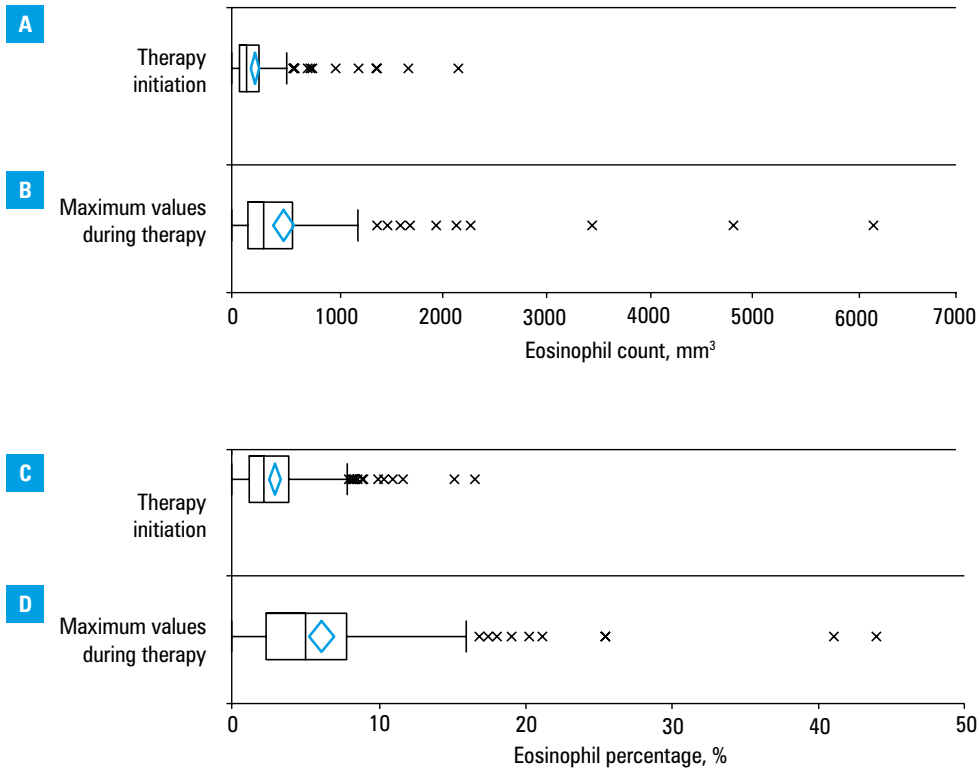

FIGURE 1 **A**, **B** – box plots of pretreatment peripheral eosinophil counts (**A**) and maximal eosinophil counts measured during the course of immune checkpoint inhibitor therapy (**B**); **C**, **D** – distribution of the percentage of pretreatment peripheral eosinophils (**C**) and the maximum eosinophil percentage measured during the course of therapy (**D**)

TABLE 3 Peripheral eosinophils in the study patients with controlled and progressive disease

Eosinophils	Controlled disease[a] (n = 135)	Progressive disease (n = 55)	*P* value
At the time of ICPI therapy initiation			
Count, n/μl	151 (97–297)	92 (62–245)	0.02
Percentage	2.4 (1.3–4)	1.9 (0.8–3.3)	0.07
During the ICPI therapy			
Maximum count, n/μl	372 (187–741)	218 (133–381)	0.002
Maximum percentage	5.9 (2.9–8.3)	3.4 (1.6–5.3)	<0.001

Data are presented as median (interquartile range). The Mann–Whitney test was used for statistical analysis.

a Patients with controlled disease included those with complete response, partial response, and stable disease.

Abbreviations: see **TABLE 1**

studies,[11,28] the maximum eosinophil count was set to 300/μl and the cutoff value, to 5%. Patients were divided into 2 groups to examine TTF. Regarding eosinophil counts and percentages at the time of ICPI therapy initiation, a significant difference in TTF between the 2 groups was observed, when the cutoff values were set to 150/μl (P = 0.046) and 3% (P = 0.03), respectively. As for the maximum eosinophil counts and percentages during ICPI therapy, a significant difference in TTF between the 2 groups was noted, when the cutoff values were set to 150/μl (P <0.001), 300/μl (P <0.001), 3% (P <0.001), and 5% (P <0.001), respectively (**FIGURE 3**). The multivariable analysis of TTF showed that a maximum eosinophil percentage greater than 5% during ICPI therapy was a significant predictive indicator for prolonged therapeutic efficacy, as were "good performance status" and "PD-L1 expression level of 50% or more" (**TABLE 5**).

DISCUSSION The level of PD-L1 expression has been used as a biomarker of the efficacy of ICPI therapy. However, PD-L1 commonly exhibits a nonuniform immunostaining pattern across cancer tissue, which leads to inaccuracies in interpreting the overall expression. Numerous studies have sought to identify other biomarkers in ICPI therapy,[10,17,26,27] but there is no established biomarker other than PD-L1.[9] The identification of a biomarker that is also a routinely used clinical marker would be of value, as it may represent a convenient and low-cost option that does not require special equipment.

In our clinical practice, we observed a patient with NSCLC who responded to ICPI therapy during eosinophilia and progressed following eosinophilia normalization.[20] Based on that experience, we focused on the change in the number of peripheral eosinophils before and during ICPI therapy by investigating the relationship between peripheral eosinophil counts and percentages and the efficacy of ICPI therapy. Eosinophilia may develop in adrenal deficiency due to ICPI use.[29,30] However, according to a review by Domagała-Kulawik et al,[31] hematologic irAEs do occur very rarely. The authors noted that hematologic irAEs should be distinguished from transient changes in laboratory blood test results at the time of ICPI therapy initiation. Eosinophils play the key role in allergic and parasitic diseases. Although their role in cancer immunity

TABLE 4 Peripheral eosinophils and response to immune checkpoint inhibitor therapy in the study patients with non–small cell lung cancer

Eosinophils	CR (n = 4)	PR (n = 64)	SD (n = 67)	PD (n = 55)	*P* value
At the time of ICPI therapy initiation					
Count, n/μl	512 (347–635)	152 (88–282)	148 (104–265)	92 (62–245)	0.02
Percentage	7.5 (5.8–7.9)	2.4 (1.3–3.8)	2.2 (1.4–3.9)	1.9 (0.8–3.3)	0.09
During the ICPI therapy					
Maximum count, n/μl	777 (457–1309)	437 (228–841)	244 (153–537)	218 (133–381)	<0.001
Maximum percentage	8.4 (7.2–11)	7.1 (4.1–10.3)	4.2 (2.5–6.8)	3.4 (1.6–5.3)	<0.001

Data are presented as median (interquartile range). The Kruskal–Wallis test was used for statistical analysis.

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; others, see TABLE 1

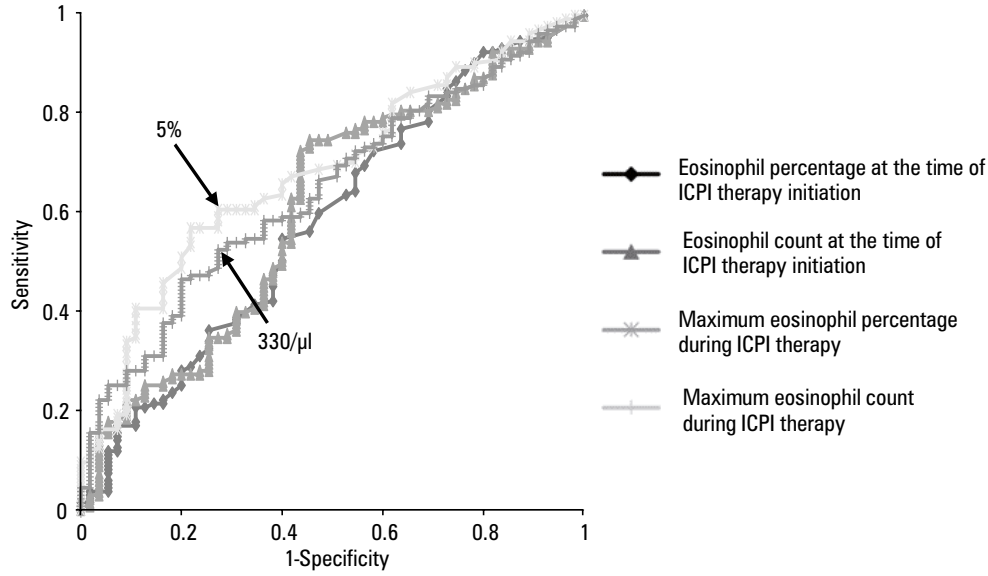

FIGURE 2 Receiver operating characteristics curves for the cutoff levels of eosinophil counts and percentages at the time of immune checkpoint inhibitor (ICPI) therapy initiation and the maximum eosinophil counts and maximum eosinophil percentages during the clinical course of immune checkpoint inhibitor therapy

was not mainstream, there have been reports on cancer patients with peripheral eosinophilia and those with eosinophil infiltration into tumors.[22-25] The mechanism and clinical significance of eosinophilia in peripheral blood and tumor tissues remains unclear, but the involvement of some cytokines has been suggested.[10,24] In recent years, ICPIs have become common drugs for the treatment of cancer in various organs and more attention is being paid to the relationship between eosinophils and cancer immunity.[10,17,26,27] In particular, peripheral eosinophils as a biomarker of ICPI therapy efficacy have been a matter of interest.[6-8,10,11,16-18,26] Simon et al,[10] who studied both basic and clinical aspects of peripheral blood eosinophils in ICPI treatment, demonstrated that response to ICPI treatment was associated with eosinophil accumulation in the peripheral blood of patients with melanoma.[10] Interestingly, they showed an association between elevated serum levels of interleukin 16 and an increase in peripheral eosinophils during ICPI therapy. Using immunohistochemistry, they also observed an enhanced eosinophil degranulation and a positive correlation between eosinophils and CD8+ T cell infiltration of tumor tissues in patients with melanoma treated with ICPIs.[10] Additionally, Umansky et al[7] reported on the involvement of myeloid-derived suppressor cells and eosinophils in the therapeutic effect of ipilimumab.[7] In patients with melanoma, for example, an elevated peripheral eosinophil count at the time of diagnosis,[8] during ipilimumab treatment,[6] and after initial ICPI therapy[10] were associated with a favorable prognosis. The relationship between peripheral eosinophils and the therapeutic effect of ICPI use was investigated not only in patients with melanoma but also in those with NSCLC[11,13,16] and Hodgkin lymphoma.[19] Regarding patients with NSCLC, there have been reports focused on eosinophilia before ICPI use.[11,12] Tanizaki et al[11] used CBC measured within 7 days before ICPI therapy initiation.[11] On the other hand, some studies have concentrated on the increase following ICPI therapy initiation.[10,16] Simon et al[10]

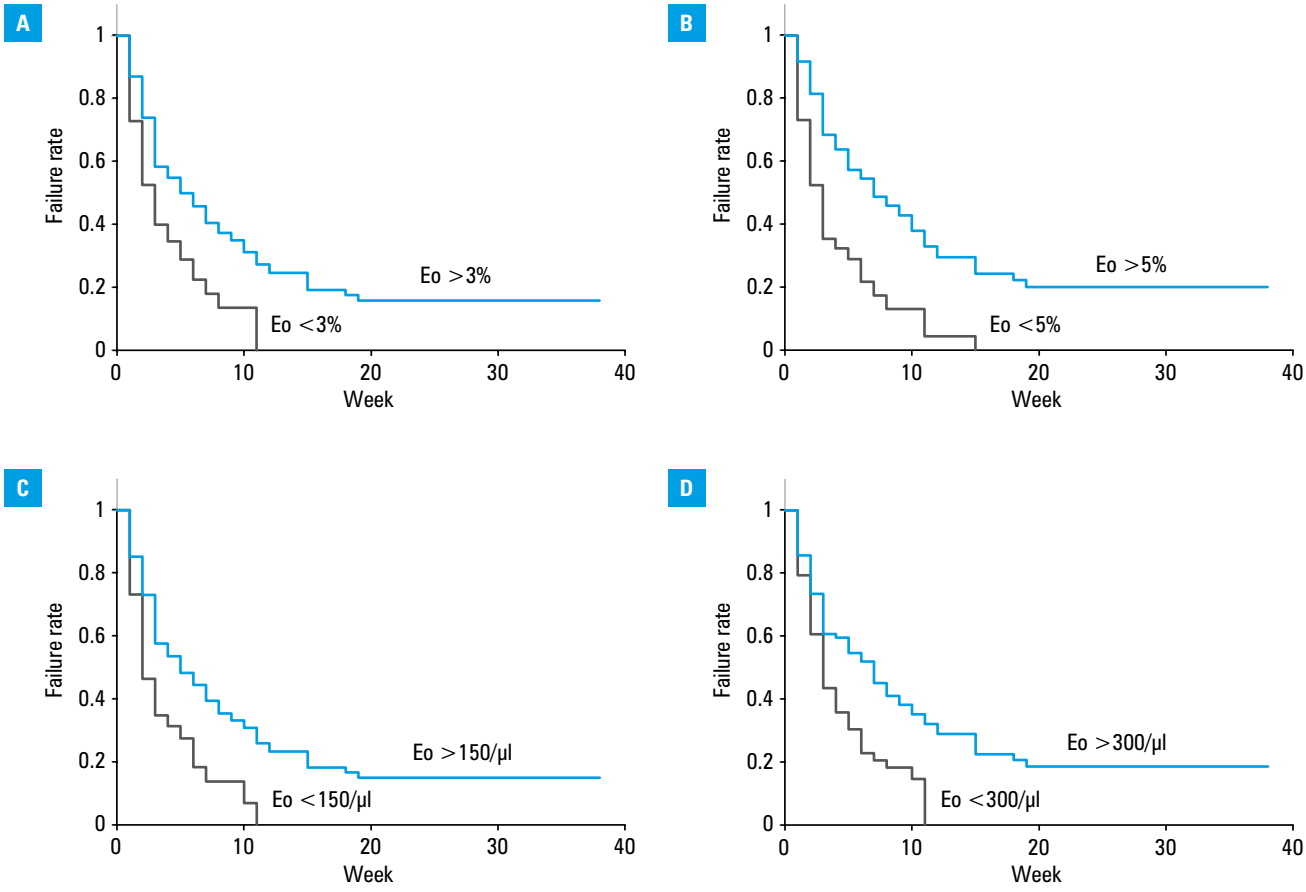

FIGURE 3 Time to treatment failure curves with a cutoff value for eosinophil (Eo) percentage of 3% (**A**) and 5% (**B**) and for eosinophil count of 150/μl (**C**) and 300/μl (**D**) during immune checkpoint inhibitor therapy

TABLE 5 Results of multivariable analysis using the Cox proportional hazards model

Factor	Exp	95% CI	*P* value
PS (ECOG)	1.5112	1.1806–1.9344	0.001
PD-L1 ≥50%	0.6549	0.4326–0.9914	0.0454
Maximum eosinophil percentage ≥5%	0.3978	0.2630–0.6016	<0.001

The forward-backward stepwise method was used for the choice of independent variables used in the Cox proportional hazards model.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; Exp, exponential function; PD-L1, programmed cell death ligand 1; others, see **TABLE 1**

evaluated peripheral blood drawn 12 to 32 days after the first administration of ICPIs.[10] However, it is not clear at which timepoint, before or after ICPI therapy, peripheral eosinophilia should be deemed relevant. If the change in eosinophils after ICPI therapy initiation is of clinical significance, there also appears the question as to when eosinophilia should be considered important. In addition, it remains unknown which parameter is of greater importance—eosinophil counts or percentages—and what is the optimal cutoff value for each of them.

In the present study, we observed an increase in both peripheral eosinophil counts and eosinophil percentages after ICPI therapy initiation, regardless of whether the patients had controlled or progressive disease. The median time to note the maximum eosinophil percentage was 5 weeks in patients with controlled disease. The ROC curve analysis showed that the cutoff values for patients with controlled disease and those with progressive disease were an eosinophil count of 330/μl and an eosinophil percentage of 5%, where an eosinophil count of 300/μl may be of use in clinical practice. We observed a significant difference in TTF between patients stratified by these cutoff values. Finally, we identified eosinophil percentages greater than 5% during ICPI therapy as a significant predictive factor for prolonged therapeutic efficacy in a multivariable analysis. The results of this study suggest that it may be possible to predict response to ICPI therapy from peripheral eosinophils. Based on the obtained findings, we observed that changes in eosinophils after treatment were more significant than those at the pretreatment stage. Furthermore, we determined that changes in eosinophils noted within 5 weeks of ICPI therapy initiation were of greatest importance.

Although clinically significant changes in eosinophil levels has been considered a potential biomarker for eosinophilia, there have been different views on the significance of peripheral eosinophila. In other words, its importance as a biomarker depended on whether it exhibited therapeutic responsiveness, long-term progression-free

survival, or good prognosis. Its significance was generally regarded as a feature of a favorable marker. On the other hand, there have been reports on patients who developed eosinophilia syndrome with unfavorable complications.[32,33] Considering those complications, we hypothesized that eosinophilia after starting treatment is an indicator of cancer immunity initiation.

Admittedly, this study had several limitations. First, it was a retrospective study that included patients with various baseline characteristics. Second, it involved a limited number of patients with a short follow-up, and the number of patients required was not preset on a statistical basis. Third, we did not elucidate the relationship between changes in eosinophils following ICPI therapy and the biological role of eosinophils. Fourth, in this study, there was no significant difference in the patients' baseline characteristics stratified by treatment regimens, so all patients were analyzed together. However, it was considered important to investigate the change of eosinophils according to treatment methods used. In particular, cytotoxic anticancer drugs can affect peripheral blood cells, so it may be necessary to analyze patients receiving them separately. We showed that ICPI therapy was effective in patients who had increased eosinophil counts and elevated eosinophil percentages. Yet, it remains unclear whether this therapeutic effect weakened consistently with the decreasing eosinophil count and percentage. This issue could not be solved. It also should be clarified whether a temporary or continuous increase in eosinophils is of importance. Our team is currently examining this issue. The latter seems to have a better prognosis so far (data not shown).

It would not be difficult or expensive to include the measurement of peripheral eosinophils when measuring CBC and investigate the association of eosinophil counts and percentages with the efficacy of ICPI therapy. Along these lines, we are now planning a sufficiently powered large-scale study with a higher number of patients to confirm the findings from this study.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

CONTRIBUTION STATEMENT SO and HS designed the study. SO, TS, KM, YS, GO, KK, SS, TK, and HS collected the data. SO, KN, HS, and NH analyzed the data and prepared the manuscript. All authors approved the final version of the article.

CONFLICT OF INTEREST None declared.

HOW TO CITE Okauchi S, Shiozawa T, Miyazaki K, et al. Association between peripheral eosinophils and clinical outcomes in patients with non–small cell lung cancer treated with immune checkpoint inhibitors. Pol Arch Intern Med. 2021; 131: 152-160. doi:10.20452/pamw.15776

REFERENCES

1 Remon J, Passiglia F, Ahn MJ, et al. Immune checkpoint inhibitors in thoracic malignancies: review of the existing evidence by an IASLC Expert Panel and recommendations. J Thorac Oncol. 2020; 15: 914-947.

2 Lantuejoul S, Sound-Tsao M, Cooper WA, et al. PD-L1 testing for lung cancer in 2019: perspective from the IASLC pathology committee. J Thorac Oncol. 2020; 15: 499-519.

3 Williams JB, Li S, Higgs EF, et al. Tumor heterogeneity and clonal cooperation influence the immune selection of IFN-γ-signaling mutant cancer cells. Nat Commun. 2020; 11: 602.

4 Wang X, Zhang B, Chen X, et al. Lactate dehydrogenase and baseline markers associated with clinical outcomes of advanced esophageal squamous cell carcinoma patients treated with camrelizumab (SHR-1210), a novel anti-PD-1 antibody. Thorac Cancer. 2019; 10: 1395-1401.

5 Benitez JC, Recondo G, Rassy E, Mezquita L. The LIPI score and inflammatory biomarkers for selection of patients with solid tumors treated with checkpoint inhibitors. Q J Nucl Med Mol Imaging. 2020; 64: 162-174.

6 Delyon J, Mateus C, Lefeuvre D, et al. Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. Ann Oncol. 2013; 24: 1697-1703.

7 Umansky V, Utikal J, Gebhardt C. Predictive immune markers in advanced melanoma patients treated with ipilimumab. Oncoimmunology. 2016; 5: e1158901.

8 Moreira A, Leisgang W, Schuler G, Heinzerling L. Eosinophilic count as a biomarker for prognosis of melanoma patients and its importance in the response to immunotherapy. Immunotherapy. 2017; 9: 115-121.

9 Buder-Bakhaya K, Hassel JC. Biomarkers for clinical benefit of immune checkpoint inhibitor treatment – a review from the melanoma perspective and beyond. Front Immunol. 2018; 9: 1474.

10 Simon SCS, Hu X, Panten J, et al. Eosinophil accumulation predicts response to melanoma treatment with immune checkpoint inhibitors. Oncoimmunology. 2020; 9: 1727116.

11 Tanizaki J, Haratani K, Hayashi H, et al. Peripheral blood biomarkers associated with clinical outcome in non-small cell lung cancer patients treated with nivolumab. J Thorac Oncol. 2018; 13: 97-105.

12 Facchinetti F, Veneziani M, Buti S, et al. Clinical and hematologic parameters address the outcomes of non-small-cell lung cancer patients treated with nivolumab. Immunotherapy. 2018; 10: 681-694.

13 Fujimoto S, Fujita A, Minato K, et al. Complete response of a patient with lung squamous cell carcinoma after only three administrations of nivolumab [in Japanese]. Jpn J Lung Cancer (Haigan). 2018; 58: 292-297.

14 Inomata M, Kado T, Okazawa S, et al. Peripheral PD1-positive CD4 T-lymphocyte count can predict progression-free survival in patients with non-small cell lung cancer receiving immune checkpoint inhibitor. Anticancer Res. 2019; 39: 6887-6893.

15 Soda H, Ogawara D, Fukuda Y, et al. Dynamics of blood neutrophil--related indices during nivolumab treatment may be associated with response to salvage chemotherapy for non-small cell lung cancer: A hypothesis-generating study. Thorac Cancer. 2019; 10: 341-346.

16 Lou Y, Marin-Acevedo JA, Vishnu P, et al. Hypereosinophilia in a patient with metastatic non-small-cell lung cancer treated with antiprogrammed cell death 1 (anti-PD-1) therapy. Immunotherapy. 2019; 11: 577-584.

17 Alves A, Sucena I, Dias M, et al. Eosinophilia in lung cancer patients treated with immunotherapy. Eur Respir J. 2019; 54: PA4664.

18 Singh N, Lubana SS, Constantinou G, Leaf AN. Immunocheckpoint inhibitor- (Nivolumab-) associated hypereosinophilia in non-small-cell lung carcinoma. Case Rep Oncol Med. 2020: 7492634.

19 Hude I, Sasse S, Bröckelmann PJ, et al. Leucocyte and eosinophil counts predict progression-free survival in relapsed or refractory classical Hodgkin lymphoma patients treated with PD1 inhibition. Br J Haematol. 2018; 181: 837-840.

20 Osawa H, Okauchi S, Taguchi S, et al. Immuno-checkpoint inhibitor--associated hyper-eosinophilia and tumor shrinkage. Tuberk Toraks. 2018; 66: 80-83.

21 Klion AD, Ackerman SJ, Bochner BS. Contributions of eosinophils to human health and disease. Annu Rev Pathol. 2020; 15: 179-209.

22 Sawyers CL, Golde DW, Quan S, Nimer SD. Production of granulocyte--macrophage colony-stimulating factor in two patients with lung cancer, leukocytosis, and eosinophilia. Cancer. 1992; 69: 1342-1346.

23 Matsumoto S, Tamai T, Yanagisawa K, et al. Lung cancer with eosinophilia in the peripheral blood and the pleural fluid. Intern Med. 1992; 31: 525-529.

24 Pandit R, Scholnik A, Wulfekuhler L, Dimitrov N. Non–small-cell lung cancer associated with excessive eosinophilia and secretion of interleukin-5 as a paraneoplastic syndrome. Am J Hematol. 2007; 82: 234-237.

25 El-Osta H, El-Haddad P, Nabbout N. Lung carcinoma associated with excessive eosinophilia. J Clin Oncol 2008; 26: 3456-3457.

26 Rigoni A, Colombo MP, Pucillo C. Mast cells, basophils and eosinophils: from allergy to cancer. Semin Immunol. 2018; 35: 29-34.

27 Simon SCS, Utikal J, Umansky V. Opposing roles of eosinophils in cancer. Cancer Immunol Immunother. 2019; 68: 823-833.

28 Yilmaz I, Turk M. What should be the cutoff value of blood eosinophilia as a predictor of inhaled corticosteroid responsiveness in patients with chronic obstructive pulmonary disease? Am J Respir Crit Care Med. 2017; 196: 1229-1230.

29 Ariyasu H, Inaba H, Ota T, et al. Thyrotoxicosis and adrenocortical hormone deficiency during immune-checkpoint inhibitor treatment for malignant melanoma. In Vivo. 2018; 32: 345-351.

30 Ariyasu R, Horiike A, Yoshizawa T, et al. Adrenal insufficiency related to anti-programmed death-1 therapy. Anticancer Res. 2017; 37: 4229-4232.

31 Domagała-Kulawik J, Leszek P, Owczarek W, et al. Immunotherapy of solid tumors: safety of treatment. Pol Arch Intern Med. 2020; 130: 766-778.

32 Khoja L, Maurice C, Chappell M, et al. Eosinophilic fasciitis and acute encephalopathy toxicity from pembrolizumab treatment of a patient with metastatic melanoma. Cancer Immunol Res. 2016; 4: 175-178.

33 Anastasopoulou A, Papaxoinis G, Diamantopoulos P, et al. Bullous pemphigoid-like skin lesions and overt eosinophilia in a patient with melanoma treated with nivolumab: case report and review of the literature. J Immunother. 2018; 41: 164-167.