

Association of Blood Biochemical Indexes and Antibiotic Exposure With Severe Immune-related Adverse Events in Patients With Advanced Cancers Receiving PD-1 Inhibitors

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Summary: Some patients with cancer treated with programmed death 1 (PD-1) inhibitors experience immune-related severe adverse events (ir-SAEs), however, predictors are limited. The objective was to identify clinicopathologic features that may be associated with a higher ir-SAE risk. This was a nested case-control study. After screening a total of 832 PD-1 inhibitor-treated patients, we identified 42 ir-SAE cases. According to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, ir-SAEs were defined as grade ≥ 3 toxic effects associated with immunotherapy. A total of 126 controls were matched. The crude and adjusted risks of ir-SAEs were estimated by odds ratio (ORs) and 95% CIs using multivariate logistic regression models. Baseline neutrophil-to-lymphocyte ratio (NLR) [per SD increment-adjusted (aOR): 1.16], lactate dehydrogenase (LDH) ≥ 245 U/L (aOR: 2.39), and antibiotic exposure (aOR: 4.39) were associated with a higher risk of ir-SAEs. When NLR was categorized in 3 groups, significantly higher risks of ir-SAEs (aOR: 4.95) were found in participants in group 3 (> 6) than in those in group 1 (< 3). Furthermore, NLR (per SD increment-adjusted hazard ratio: 1.08) were also significantly associated with shorter overall survival (OS). Baseline LDH ≥ 245 U/L and antibiotic exposure were no significant association with OS. In conclusion, ir-SAEs were associated between baseline NLR, LDH ≥ 245 U/L and antibiotic exposure. Lower NLR was correlated with longer OS for cancer.

Key Words: neutrophil-to-lymphocyte ratio, lactate dehydrogenase, antibiotic exposure, immune-related severe adverse events, case-control study

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In the last few years, programmed death 1 (PD-1) inhibitors have enhanced the therapeutic outcomes for patients with advanced solid tumors.¹ However, despite the better tolerance to

PD-1 inhibitors when compared with conventional chemotherapy, some patients endure substantial toxicities in many systems such as the skin, endocrine, hepatic, gastrointestinal, and respiratory systems, which are described as immune-related adverse events (irAEs).^{2–4} Severe irAEs [grade ≥ 3 according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0] may cause treatment discontinuation or death.²

ir-SAEs occur in 0.5%–13% of the patients treated with immunotherapy.⁵ Pneumonitis, hypothyroidism, and myasthenia gravis are more common with anti-PD-1 antibodies than with anti-CTLA-4 antibodies.⁶ Most of the toxicities appear temporally. The onset time from the initiation of immune-checkpoint inhibitors (ICIs) treatment is not the same for different types of adverse effects.⁵ Moreover, the median time from symptomatic onset to death in some fatal irAEs is only 32 days.⁷ Therefore, clinical biomarkers for predicting the occurrence of ir-SAEs are required for better clinical management of these outcomes. Compared with biomarkers for predicting tumor responses, biomarkers for ir-SAEs have been less investigated. These biomarkers include low muscle attenuation, serum IL-17, T-cell repertoire, gut microbiome or pre-existing autoimmune diseases.^{8,9} Moreover, clinical biomarkers, including pretreatment laboratory indices, have not been clearly elucidated.

This study aimed at investigating the demographic, clinical, and laboratory markers that are associated with higher ir-SAE risks. We hypothesized that some baseline laboratory indices and select clinical features are correlated with an increased risk of ir-SAEs during anti-PD1 treatment.

METHODS

Study Design and Participants

This study was performed at the Jiangsu Cancer Hospital. A total of 832 advanced cancer patients who had received at least 1 dose of anti-PD-1 antibodies between August 2018 and June 2020 were retrospectively analyzed. Anti-PD-1 antibodies include nivolumab, pembrolizumab, camrelizumab, and toripalimab. The exclusion criteria included patients who dropped out of treatment because of Medicare costs ($n = 155$) and those with missing baseline data ($n = 175$). The remaining cohort included 502 participants. Among them, we identified 83 SAE cases. Each SAE was independently determined by 2 medical oncologists. Finally, non-immune-related or unidentified cases were ruled out and the remaining 42 cases were selected in the study. Toxicities were graded using the CTCAE version 5.0. The ir-SAEs (grade ≥ 3) are ICI-induced autoimmune toxicities that reflect

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a disorder of the immune system and are associated with hospitalization, life-threatening situations, and death.

The control group was also obtained from the same cohort. Subsequent to enrollment of a case, eligible control cases for sex, anti-PD-1 antibody and cancer type were approached until 3 control patients were individually matched to each case-patient. Finally, 126 matched controls were included in this study (Fig. 1).

This study was ethically approved by the local Ethics Committee of Jiangsu Cancer Hospital (Jiangsu, China). An informed consent was exempt as patient data were collected anonymously.

Data Collection

Regarding initial PD-1 inhibitor treatment, clinical data including demographic data (age, sex), Eastern Cooperative Oncology Group performance status (ECOG PS), disease staging, number of metastatic sites, antibiotic exposure within 3 months (90 d) before the first ICI infusion, treatment data, occurrence of ir-SAE (including time, site/organ, severity), and time of death or last follow-up were recorded. Baseline measurements were taken within 1 week before the beginning of ICI treatment. Relevant laboratory indices, including neutrophil counts, lymphocyte counts, platelet counts, serum albumin, and lactate dehydrogenase (LDH) levels were collected for each patient. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were determined as previously described.^{10,11} NLR (absolute neutrophil count divided by absolute lymphocyte count) and PLR (absolute platelet count divided by absolute lymphocyte count).

Statistical Analysis

Continuous variables are expressed as mean ± SD or median [interquartile range (IQR)], while categorical variables are expressed by number and percentage. Proportions in baseline characteristics between cases and controls were compared using the Mann-Whitney *U* test for continuous variables, and the χ^2 test or Fisher exact test for categorical variables. Possible correlations between measured parameters and ir-SAEs were assessed using multivariate binary logistic regression model. The multivariate model comprised factors of clinical interest and a univariable screen ($P < 0.1$) in the univariate analysis. Three models were fitted: (i) model 1: adjusted for sex and age; (ii) model 2: adjusted for sex, age, ECOG PS, monotherapy, line of immunotherapy, and M stage; and (iii) model 3: additionally, adjusted for combination with anti-angiogenesis, and antibiotic exposure. Odds ratios (OR) and 95% confidence interval (CI) were accordingly calculated. NLR was also converted into a categorical variable after which *P* for trend was determined to verify the results of NLR as a continuous variable. Overall survival (OS) outcomes were calculated from the start of PD-1 inhibitor treatment until death or last follow-up. Kaplan-Meier method was used to compare nominal OS (log-rank test) between groups.

Statistical analyses were performed using the R 3.3.2 software package (<http://www.Rproject.org>, The R Foundation) and the Free Statistics software versions 1.1. $P \leq 0.05$ (2-sided) was set as the threshold for statistical significance.

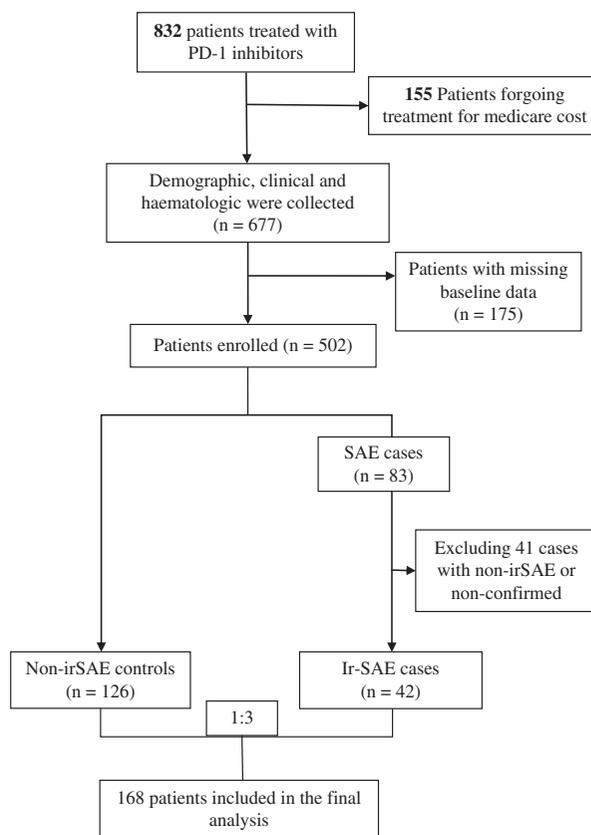


FIGURE 1. Schematic presentation for screening and enrollment of study participants. PD-1 indicates programmed death-1; Ir-SAE, immune-related severe adverse event.

RESULTS

Demographic Characteristics

A total of 168 patients with a median age of 61 years were enrolled in this case-control study. The two groups were balanced in terms of age, sex, ECOG PS, cancer type, M stage, history, and line of PD-1 inhibitor therapy. There were no significant differences in PLR levels, platelet $\geq 350 \times 10^9/L$ counts, hemoglobin < 130 g/L and albumin < 34 g/L levels. However, cases of ir-SAEs were frequent in patients with a previous antibiotic exposure ($P < 0.001$), patients with LDH ≥ 245 U/L ($P = 0.0331$), and NLR ($P = 0.011$) (Table 1).

Clinical Features of Ir-SAEs

Figure 1 shows that after screening a total of 832 individual ICI-related cases, 42 ir-SAE cases were identified. Among them (Table 2), 8(19%) cases had been administered with anti-PD-1 monotherapy, 34(81%) had been administered anti-PD-1 plus anti-angiogenesis or chemotherapy. The most common grade ≥ 3 anti-PD1 induced adverse event was pneumonitis (52%). Skin, hepatitis, and cardiac toxic effects were found in 7%–12% of the reported cases. Hypophysitis, colitis, myositis, adrenal insufficiency, neurological, hematologic, and gastrointestinal bleeding had the lowest reported prevalence (2%–5%). Among the 42 cases, 35 patients (83%) had grade 3 treatment-related adverse reactions. Grade 4 toxicities occurred in 5 patients (12%). For grade 5 toxicities, there was one case each for

TABLE 1. Baseline Characteristics for All Patients

Characteristics	N = 168 [n (%)]	Non-irSAE (N = 126)	ir-SAE (N = 42)	P
Age, median (± SD) (y)	60.8 ± 10.2	60.9 ± 10.4	60.3 ± 9.6	0.643
Male, n (%)	132 (78.6)	99 (78.6)	33 (78.6)	<1.00
ECOG PS				
0 or 1	119 (70.8)	90 (71.4)	29 (69.0)	0.922
≥ 2	49 (29.2)	36 (28.6)	13 (31.0)	
Types of cancer				<1.00
Lung cancer	100 (59.5)	75 (59.5)	25 (59.5)	
Esophagus cancer	32 (19.1)	24 (19.1)	8 (19.1)	
Gastrointestinal cancer	24 (14.3)	18 (14.3)	6 (14.3)	
Others*	12 (7.1)	9 (7.1)	3 (7.1)	
M stage				0.689
M0	30 (17.9)	20 (15.9)	10 (23.8)	
M1	138 (82.1)	106 (84.1)	32 (76.2)	
Number of metastasis				0.204
0 or 1	100 (59.5)	79 (62.7)	21 (50)	
≥ 2	68 (40.5)	47 (37.3)	21 (50)	
Line of PD-1 inhibitor therapy				0.364
1 line	46 (27.4)	31 (24.6)	15 (35.7)	0.162
≥ 2 line	122 (72.6)	95 (75.4)	27 (64.3)	
Preantibiotic exposure	34 (20.2)	17 (13.5)	17 (40.5)	<0.001
Previous radiotherapy	52 (31.0)	40 (31.7)	12 (28.6)	0.7
Previous chemotherapy	43 (25.6)	31 (24.6)	12 (28.6)	0.610
Previous targeted therapy	69 (41.1)	54 (42.9)	15 (35.7)	0.415
Mono or combination†				
Monotherapy (IO)	28 (16.7)	20 (15.9)	8 (19.0)	0.811
IO+ anti-angiogenesis	67 (39.9)	55 (43.7)	12 (28.6)	0.084
IO+ chemotherapy	107 (63.7)	84 (66.7)	23 (54.8)	0.165
IO+ radiotherapy	39 (23.2)	27 (21.4)	12 (28.6)	0.342
Type of immunotherapy				<1.00
Nivolumab	52 (31.0)	39 (31.0)	13 (31.0)	
Pembrolizumab	56 (33.3)	42 (33.3)	14 (33.3)	
Camrelizumab	40 (23.8)	30 (23.8)	10 (23.8)	
Toripalimab	20 (11.9)	15 (11.9)	5 (11.9)	
Medication number, IQR	7 (3.0–12.0)	8 (5.0–14.0)	2 (1.0–3.0)	<0.001
NLR, IQR	3.1 (2.3–4.2)	3.0 (2.3–3.8)	4.0 (2.5–6.4)	0.011
PLR, IQR	141 (115–223)	144 (121–220)	128 (95–226)	0.300
LDH (U/L)				
< 245‡	96 (57.1)	78 (61.9)	18 (42.9)	0.031
≥ 245	72 (42.9)	48 (38.1)	24 (57.1)	
Hemoglobin (g/L)				0.351
< 130‡	110 (65.5)	81 (64.2)	29 (69.0)	
≥ 130	58 (34.5)	45 (35.7)	13 (31.0)	
Albumin (g/L)				0.529
< 34‡	9 (5.4)	6 (4.8)	3 (7.1)	
≥ 34	159 (94.6)	120 (95.2)	39 (92.9)	

*Other cancer types were oral cancer(n=4), soft tissue sarcoma (n=4), and thymic carcinomas (n=4).

†Value sum to > 100% because patients could have > 1 condition.

‡Upper normal limit.

ECOG PS indicates performance status Eastern Cooperative Oncology Group; IO, immuno-oncology; IQR, interquartile range; ir-SAEs, immune-related severe adverse events; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

myocarditis, pneumonitis, and neurological events. The median number of PD-1 treatment was 2 (IQR, 1–3) in the ir-SAE group. The median time to ir-SAE onset from start of treatment was 29 days (IQR, 16–107 d).

Clinicopathologic Factors Associated With Ir-SAEs

In the univariate analysis, the anti PD-1/anti-angiogenesis combination group was associated with ir-SAEs ($P=0.087$, Table 3). Antibiotic exposure, NLR, and LDH ≥ 245 U/L (upper normal limit) were also associated with higher risk of ir-SAEs ($P < 0.001$, 0.004, 0.033, respectively). Age, ECOG PS, M stage, line of immunotherapy, and other combination groups were not associated with ir-SAEs (Table 3). Multivariate analysis showed that antibiotic

exposure [adjusted (a)OR: 4.39%–95% CI: 1.81–10.64, $P=0.001$], NLR (per SD increment-aOR: 1.16, 95% CI: 1.0–1.32, $P=0.019$), and LDH ≥ 245 U/L (aOR: 2.39, 95% CI: 1.08–5.32, $P=0.032$) were independent predictive factors for ir-SAEs.

Further Association of NLR With Ir-SAEs

There was a J-shaped association between NLR as a continuous variable and the risk of ir-SAEs (per SD increment-OR: 1.18; 95% CI: 1.01–1.34, Fig. 2). Table 4 shows that, as NLR increased in the non-adjusted model, there was an increasing risk of ir-SAEs (P for trend = 0.011). Participants with a higher NLR in group 3 (NLR > 6) versus group 1 (NLR < 3) had a fourfold increased risk of ir-SAEs

TABLE 2. Spectrum of Severe Immune-related Adverse Events

Variables	No. Patients (%) (n = 42)
Mono or combination	
Anti-PD-1	8 (19.0)
Combination*	34 (81.0)
Type of ir-SAE	
Pneumonitis	22 (52.4)
Hepatitis	4 (9.5)
Hypophysitis	2 (4.8)
Cardiac	3 (7.1)
Colitis	2 (4.8)
Myositis	1 (2.4)
Adrena	1 (2.4)
Neurological	1 (2.4)
Hematologic	1 (2.4)
Skin	5 (11.9)
Gastrointestinal bleeding	1 (2.4)
CTCAE grade	
Grade 3	35 (83.4)
Grade 4	4 (9.5)
Grade 5	3 (7.1)
Type of immunotherapy	
Nivolumab	13 (31.0)
Pembrolizumab	14 (33.3)
Camrelizumab	10 (23.8)
Toripalimab	5 (11.9)
Medication number	2
Median time to ir-SAE, IQR (d)	29 (16-107)

*Combination therapy with chemotherapy, radiotherapy, or antiangiogenesis agents.

CTCAE indicates Common Terminology Criteria for Adverse Events; IQR, interquartile range; ir-SAEs, immune related-severe adverse events; PD-1, programmed death-1.

(OR: 4.44; 95% CI: 1.62–12.14). After adjusting for age, sex, ECOG PS, monotherapy, line of immunotherapy, M stage, IO+ anti-angiogenesis, antibiotic exposure and LDH ≥ 245 U/L, aORs were 1.33 (95% CI: 0.53–3.29) and 4.95 (95% CI: 1.51–16.24) for NLR 3–6 and NLR > 6, respectively (*P* for trend = 0.017).

Clinicopathologic Factors Associated With OS

To further tested whether the clinicopathologic factors associated with ir-SAEs were also associated with OS, the

median follow-up duration was 15 months (95% CI: 14.7–16.5). Multivariable Cox regression analysis showed that higher baseline NLR level as a continuous variable were significantly associated with shorter OS [per SD increment-adjusted hazard ratio (aHR): 1.08, 95% CI: 1.02–1.15, *P* = 0.009; Table 5)] Compared with participants in group 1 (NLR <3), a stronger correlation with OS was found in participants in group 2 (NLR 3–6; aHR: 1.71, 95% CI: 1.09–2.66), group 3 (NLR > 6; aHR: 2.18, 95% CI: 1.17–4.08, *P* for trend = 0.004; Table 4). However, there was no significant association between baseline LDH ≥ 245 U/L and OS (aHR: 1.00, 95% CI: 0.67–1.48, *P* = 0.924; Table 4) or between antibiotic exposure and OS (aHR: 1.45, 95% CI: 0.89–2.37, *P* = 0.138; Table 5). In addition, patients who developed ir-SAEs had a decreased median OS when compared with patients without ir-SAEs (15 vs. 3 mo, log-rank *P* < 0.0001; Fig. 3). In the multivariate analysis of OS, the association between ir-SAEs and OS was also significant (aHR: 6.98, 95% CI: 4.3–11.3, *P* < 0.001) and was independent of age, sex, ECOG PS, prior lines of treatment, NLR, LDH ≥ 245 U/L and antibiotic exposure.

DISCUSSION

This study evaluated the correlation between specific laboratory biomarkers, as well as a previous history of medication with the development of severe irAEs. The risk of ir-SAEs was significantly correlated with a higher NLR, and LDH ≥ 245 U/L. A J-shaped association between NLR and the risk of ir-SAEs was observed, further confirming the relationship between the risk ratio of NLR and ir-SAEs. In addition, antibiotic exposure within 3 months before the first PD-1 antibody administration were also identified as an independent factor associated with ir-SAEs. This study provides some new information regarding antibiotic exposure and ir-SAE risk.

In this study, the most common severe adverse reaction found was pneumonitis (52%) in various cancers. The reason may be due to a high proportion of lung cancer cases (60%). Pneumonitis is a toxicity of variable onset and clinical-pathologic appearances, which is more common in patients treated PD-1 inhibitors.² Two large prospective studies reported that the incidence of grade ≥ 3 pneumonitis was similar across different tumor types, but there were more

TABLE 3. Univariate and Multivariate Analysis for Ir-SAEs

Variables		Univariate Analysis		Multivariate Analysis	
		OR (95% CI)	<i>P</i>	aOR* (95% CI)	<i>P</i>
Age	< 65 vs. ≥ 65	0.99 (0.96–1.03)	0.707		
ECOG PS	0 or 1 vs. ≥ 2	1.12 (0.52–2.40)	0.769		
M stage	M0 vs. M1	0.60 (0.26–1.42)	0.248		
Number of metastasis	0 or 1 vs. ≥ 2	1.68 (0.83–3.40)	0.149		
Line of immunotherapy	1 vs. ≥ 2	0.59 (0.28–1.24)	0.164		
IO+ anti-angiogenesis	no vs. yes	0.52 (0.24–1.10)	0.087	0.60 (0.26–1.40)	0.237
IO+ chemotherapy	no vs. yes	0.61 (0.30–1.23)	0.167		
IO+ radiotherapy	no vs. yes	1.47 (0.66–3.24)	0.344		
Antibiotics exposure	no vs. yes	4.36 (1.81–9.71)	< 0.001	4.39 (1.81–10.64)	0.001
NLR	Per SD increment	1.19 (1.06–1.34)	0.004	1.16 (1.03–1.32)	0.019
LDH (U/L)	< 245 vs. ≥ 245†	2.17 (1.07–4.40)	0.033	2.39 (1.08–5.32)	0.032

*Adjusted for age, sex, ECOG PS, monotherapy, line of immunotherapy, and M stage, and IO+ anti-angiogenesis.

†Upper normal limit.

CI indicates confidence interval; ECOG PS, performance status Eastern Cooperative Oncology Group; IO, immuno-oncology; ir-SAEs, immune-related severe adverse events; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio.

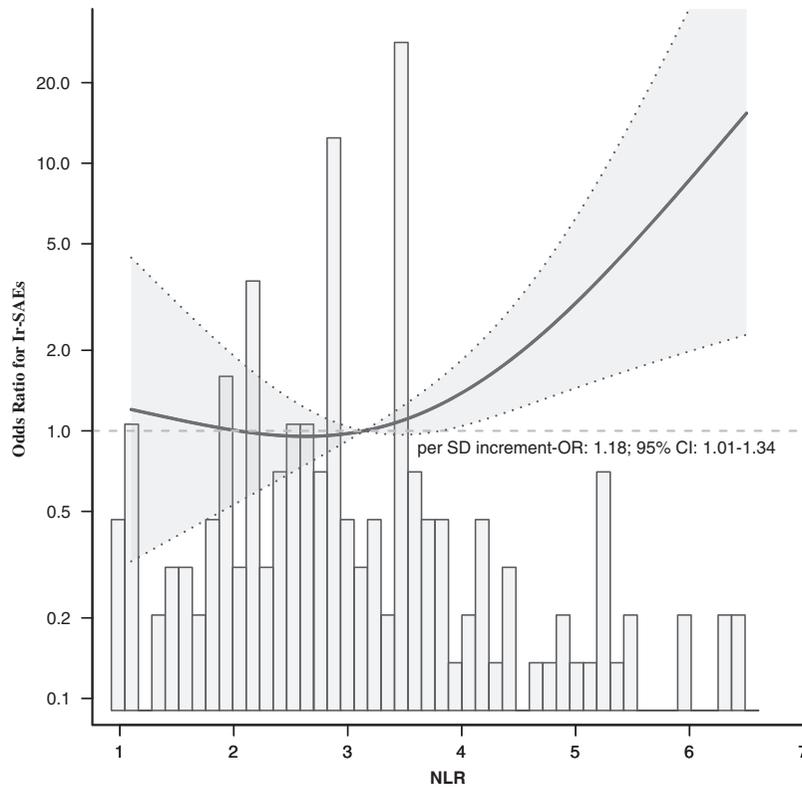


FIGURE 2. The relation of baseline NLR with the risk of ir-SAEs (per SD increment—OR: 1.18; 95% CI: 1.01–1.34). ORs of ir-SAEs were estimated by modeling NLR as a continuous variable using conditional logistic regression. Adjusted for age, sex, ECOG PS, monotherapy, line of immunotherapy, M stage, IO+ anti-angiogenesis, antibiotic exposure and LDH ≥ 245 U/L. CI indicates confidence interval; ir-SAE, immune-related severe adverse events; NLR, neutrophil-to-lymphocyte ratio.

treatment-related deaths in patients with non-small cell lung cancer (NSCLC).^{12,13} Moreover, we also observed that the median time to ir-SAE onset from start of treatment was 29 days after the second dose. The majority of adverse reaction appear temporally, with immune-related pneumonitis the earliest to appear at 8–14 weeks after treatment initiation.¹⁴ Immune-related hepatitis appears 12–16 weeks after the third dose.⁵ An influential meta-analysis reported that median time from symptom onset to death was 32 days in patients treated with ICIs. Our results are consistent with literature data. Notably, we observed that the earliest time from the first dose of PD-1 inhibitor to symptom onset was only 6 hours in 1 case. Hence, response to immunotherapy treatment should be closely monitored.

The relationship between NLR, irAE development and outcomes has been reported in several studies.^{15–18} In a recent study of patients with various cancers receiving ICI treatment, a low level of NLR (<5.3) was significantly associated with development of irAEs and longer OS.¹⁵ In NSCLC patients treated with anti-PD-(L)1 antibodies, NLR <3 was significantly correlated with irAEs.¹⁶ In addition, a different cohort study revealed that pretreatment with NLR ≥ 5 was associated with worse OS outcomes in patients with NSCLC treated with nivolumab.¹⁷ Our study partially coincided with prior studies that found that a low level of NLR (<3) is positively correlated with longer OS outcomes. It is worth noting that all of these studies were conducted in irAEs only. Our result showed that pretreatment higher NLR was

TABLE 4. Association Between NLR* and Incident Ir-SAEs

	Not Adjusted OR (95% CI)	Model I aOR (95% CI)	Model II aOR (95% CI)	Model III aOR (95% CI)
NLR	1.19 (1.06–1.34)	1.19 (1.06–1.34)	1.19 (1.06–1.35)	1.18 (1.01–1.34)
NLR groups				
G1 (<3)	1	1	1	1
G2 (3–6)	1.12 (0.5–2.51)	1.14 (0.5–2.59)	1.21 (0.52–2.77)	1.33 (0.53–3.29)
G3 (>6)	4.44 (1.62–12.14)	4.72 (1.7–13.11)	4.91 (1.63–14.78)	4.95 (1.51–16.24)
P for trend	0.011	0.009	0.013	0.017

*ORs of ir-SAEs were estimated by modeling NLR as a continuous variable and as 3 groups using multivariate logistic regression models. Model I adjusted for age and sex; model II adjusted for factors in model I + ECOG PS, monotherapy, line of immunotherapy, and M stage; model III adjusted for factors in model II + IO+ anti-angiogenesis, antibiotics exposure and LDH ≥ 245 U/L.

CI indicates confidence interval; ir-SAEs, immune related-severe adverse events; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio.

TABLE 5. Association Between Clinicopathologic Factors With OS

	HR (95%CI)	P value	aHR* (95%CI)	P value
NLR				
Per SD increment	1.09 (1.03-1.16)	0.005	1.08 (1.02-1.15)	0.009
NLR groups				
G1 (<3)	Reference		Reference	
G2 (3–6)	1.55 (1.01–2.38)	0.044	1.71 (1.09–2.66)	0.019
G3 (>6)	1.86 (1.03–3.35)	0.040	2.18 (1.17–4.08)	0.014
P for trend	0.017		0.004	
Antibiotic exposure	1.48 (0.92–2.39)	0.104	1.45 (0.89–2.37)	0.138
LDH ≥245 U/L†	1 (0.67–1.48)	0.997	1.02 (0.67–1.56)	0.924

*Adjusted for age, sex, ECOG PS, monotherapy, line of immunotherapy, and M stage, and IO+ anti-angiogenesis.

†Upper normal limit.

CI indicates confidence interval; HR, hazard ratio; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival.

positively correlated with higher severe irAE odds, and there was a J-shaped relationship between NLR and ir-SAE. This finding may suggest that, NLR >6 may reflect an auto-immune state that may result not only in development of severe immune-related toxicities but also poor efficacy and significant mortality.¹⁹ In addition, NLR <3 might trigger low-grade adverse effects, thereby enhancing anti-tumor immune responses. Patients with tolerable irAEs while on ICI therapy have been shown to have a good prognosis.

In this study, LDH ≥245 U/L was correlated with ir-SAEs. Elevated serum LDH level reflects increased tumor activity and tumor necrosis in high tumor burden disease.²⁰ Inhibition of LDH may reduce cancer cell proliferation and tumor growth.²¹ LDH has been associated with adverse outcomes in NSCLC or melanoma patients treated with ICIs.^{22–24} Previous studies^{18,23,25} reported that baseline LDH ≥240 U/L levels are associated with a shorter survival time and poor prognosis when compared with normal LDH levels. However, the relationship between LDH and the risk of irAEs has not been reported. Although we found a correlation between LDH and the risk of ir-SAEs, more studies should be performed to confirm this conclusion.

In addition to laboratory indicators, we also observed that antibiotic exposure within 3 months before the first PD-1 antibody administration was associated with an increased rate of severe immune-mediated toxicities. Gut microbiome

is an important biomarker for ICI responses in melanoma patients.¹⁹ Antibiotics may break the balance between the microbiome and the immune system, thereby influencing anti-PD1 immunotherapeutic efficacy.²⁶ Mohiuddin et al²⁷ reported that exposure of advanced melanoma patients to antibiotics before ICI had significantly poor OS outcomes than unexposed groups, and antibiotic exposure was associated with a greater incidence of immune-mediated colitis. Various studies^{28–30} have also reported that antibiotic-exposed patients have worse ICI prognosis than unexposed patients. This study supports the disruptive effects of antibiotics. However, due to our small sample size, we could not perform subgroup analysis.

Mechanisms of immune-related toxicities have not been fully elucidated. Distinct immunopathogenic mechanisms have distinct histopathological phenotypes in each target site/organ.³¹ The development of irAEs can be affected by tumor site, type and/or host microbiota. T cells are crucial in the immunopathogenesis of most irAEs.^{8,9,32} The loss of T cell tolerance can result in numerous self-directed immune processes, and the production of antibodies by activated B-cells are also plausible.³¹ Excess neutrophil activation can contribute to tissue damage during various autoimmune and inflammatory diseases.³³ Therefore, imbalancing the spectrum of immune activation is a crucial step in the development of irAEs. Alterations in NLR is associated with the occurrence of irAEs in ICIs-based therapy.

This study has some limitations. First, owing to the low incidence of ir-SAEs, the number of cases examined was relatively small and subgroup analysis was not possible. Second, it was a single-center, retrospective study. A cause-and-effect relationship cannot be inferred from a single study. Third, NLR was only analyzed at baseline, and we did not monitor the levels postintervention. Finally, patients were administered with a single agent and/or combination of anti-angiogenesis or chemotherapy for various cancer types, suggesting greater diversity and heterogeneity in the study population. Although a range of covariates were adjusted in the regression models, residual confounders due to incompletely measured factors cannot be ruled out. More studies should be performed to verify our conclusions and to explore the underlying mechanisms.

In conclusion, baseline higher NLR, LDH ≥245 U/L, and antibiotic exposure are independent risk factors for ir-SAEs in patients with cancer, and higher NLR is also associated with worse survival. Physicians should be aware of and monitor these potentially risk factors in patients receiving ICI therapy. If problems are detected, timely adjustments of treatment are necessary. There are still many clinical questions to be addressed, including whether the use of Granulocyte colony-stimulating factor will increase the rates of irAE by elevated NLR. Further immunopathogenic studies are also needed to clarify the relationship between antitumor and autoimmunity.

CONFLICTS OF INTEREST/FINANCIAL DISCLOSURES

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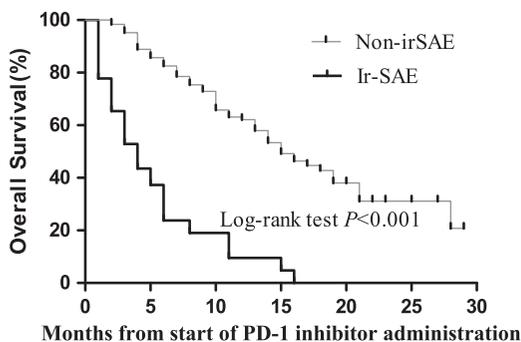


FIGURE 3. Kaplan-Meier overall survival estimate curves for development or no development of immune-related severe adverse events (irSAE) in all cancer type patients treated with anti-PD-1 antibodies.

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