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Analysis and evaluation of factors contributing to the occurrence of immune-related adverse events with immune checkpoint inhibitors

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In recent years, there has been a growing trend in the use of immune checkpoint inhibitors (ICIs) for treating a larger patient population. However, it is important to note that immune-related adverse events (irAEs) frequently arise as a result. Therefore, precise patient monitoring becomes essential. We present the findings of a retrospective study conducted at the International University of Health and Welfare Narita Hospital (referred to as "our hospital") that aimed to identify risk factors linked to the occurrence of irAEs. The study focused on analyzing various factors, including therapeutic and lifestyle backgrounds, as well as laboratory values of patients who received ICI treatment and were subsequently diagnosed with irAE. The study included patients who met the eligibility criteria for ICIs (both single agent and combination therapy) as well as ICI in combination with anticancer drugs. The inclusion period for the study encompassed April 2020 to May 2022 at our hospital. The fifty patients were divided into two groups based on the severity of irAEs: the first group consisted of patients with irAE Grade 2 or lower (referred to as irAE Grade under 2), while the second group included patients with irAE Grade 3 or higher (referred to as irAE Grade over 3). Statistical analysis revealed significant differences in age (p=0.027) and CRP (C-reactive protein) levels (p=0.008) among the background factors when comparing the two groups. Additionally, statistically significant differences were observed among different ICI treatment groups in the occurrence of irAEs (p=0.035). however, it was indicated to be a relatively weak correlation. Moving forward, we shifted our focus to examine the frequency of irAEs in relation to exposure. However, we did not observe any significant correlation between exposure and irAE grade. Additionally, even when exposure was doubled through the use of ipilimumab in combination with ICIs (referred to as "Mod exposure"), no correlation was found. Exposure was further categorized into three groups: the PD-1 group, PD-L1 group, and PD-1 + CTLA-4 group. However, no significant correlation was observed between exposure in any of these groups and the grade of irAEs. Similarly, no significant correlation was observed between the dosage of ICI in the fixed-dose group and the weight-based dosage group with exposure and irAE Grade.
Based on our study findings, there is a suggestive relationship between age and CRP levels and the occurrence of irAEs of Grade 3 or higher. These factors may play a role in contributing to the development of more severe irAEs.

1. Introduction

In recent times, immune checkpoint inhibitors (ICIs) have been increasingly recommended for the treatment of malignant melanoma and unresectable advanced or recurrent non-small cell lung cancer, among others. However, as the occurrence of immune-related adverse events (irAEs) is not uncommon and can sometimes lead to serious side effects, accurate monitoring of patients is essential.

Among irAEs, liver dysfunction and endocrine disorders can often be detected and managed early through blood test results. However, Myasthenia gravis and myositis are more challenging to identify solely based on patient symptoms, which may result in serious side effects. Therefore, there is a strong need for evidence or methods to predict and prevent such serious side effects, which are eagerly awaited in the medical community.

Indeed, there have been reports indicating that the types and frequencies of irAEs vary between ICIs that target different molecules (Postow et al. 2018). Additionally, the frequency of irAEs can also differ depending on whether ICIs are administered as single agents or in combination with other therapies (Larkin et al. 2015). These differences highlight the importance of understanding the specific characteristics and risks associated with different ICIs to ensure accurate patient monitoring and appropriate management of irAEs.

Furthermore, the Eastern Cooperative Oncology Group's (ECOG) systemic status, commonly measured by performance status, and smoking history are recognized as risk factors for lung-related irAEs (Okada et al. 2020). However, there have been limited reports that comprehensively evaluate the association between patient background factors and the occurrence of irAEs. More research is needed in this area to better understand and identify additional risk factors that may contribute to the development of irAEs in different patient populations.

In the study by Shulgin et al. (2020), as one method, exposure (Eq. 1) was defined as the steady-state average plasma concentration

(mg/L) divided by the 50% inhibitory concentration (mg/L). This measure was used to compare and evaluate various ICI treatments. To analyze the incidence rates of irAEs, they employed a generalized linear mixed effects model (GLMM) to develop a robust inference model. This approach allowed them to better understand and compare the impact of different ICIs on irAE rates, taking into account exposure levels and other relevant factors.

While there have been speculative models developed for some ICIs such as nivolumab, pembrolizumab and ipilimumab, the same level of research and models for atezolizumab, durvalumab and avelumab, which are now more commonly used, has been lacking. These latter ICIs are gaining popularity in clinical practice, but their specific relationships with irAE incidence rates and exposure have not been extensively studied yet. Further research is needed to develop more comprehensive and accurate models for these drugs to guide their clinical use and management of irAEs.

The objective of this study is to conduct a cross-sectional correlation analysis between exposure to ICIs, patient background, and irAEs. The primary focus is on correlating the occurrence of irAEs with exposure to specific ICIs. Additionally, the study aims to investigate the correlation between irAE incidence and exposure to other chemotherapy agents in combination with ICIs. By conducting this covariate exploration, researchers seek to gain insights into the relationship between different treatments and irAE rates, thereby improving patient monitoring and management strategies in clinical practice.

Indeed, given that the model proposed by Shulgin et al. for the dependence of irAE rates on ICI dose/exposure lacks the ability to specifically estimate the incidence of irAEs with exposure to PD-1 (programmed death receptor-1) targeting drugs alone, our study aims to develop a more comprehensive model. The objective is to construct a model for the dependence of irAE rates on ICI dose/exposure that can include PD-1 targeting drugs as standalone treatments. By doing so, we aim to provide a more accurate and complete understanding of irAE incidence rates related to PD-1 inhibitors and their exposure, which can be valuable in clinical decision-making and patient care.

In this research, we present findings from a retrospective study carried out at the International University of Health and Welfare Narita Hospital, referred to as “our hospital.” The main aim of this study is to identify risk factors linked to the development of irAEs in patients undergoing ICI treatment. We focused on analyzing various individual factors, including therapeutic and lifestyle

Table 1: Patient characteristics

Characteristics	Patients (n=50)
Age (years) median (range)	71 (37-89)
Gender	
Male / Female	39 / 11
Hight (cm) median (range)	164.5 (139.0-193.2)
Body weight (kg) median (range)	58.7 (40.3-109.0)
ECOG PS	
0 / 1 / 2 / 3	37 / 9 / 3 / 1
Clinical Stage	
/ / Postoperative relapse	10 / 27 / 13
Cancer type	
Lung	29
Stomach	5
Uroepithelium	3
Pleural mesothelium	2
Liver	5
Others	6
Types of ICI	
ICI	31
ICI+IPI	9
ICI+Chemo	23
ICI+IPI+Chemo	4
Metastatic organs	
0 / 1 / 2 / 3	24 / 19 / 4 / 3
Treatment line	
1st / 2nd / 3rd / Greater	26 / 12 / 4 / 8
Smoking status	
Current, Former / Never / Unknown	41 / 5 / 4
History of steroid use	
Yes / No	28 / 22
History of antibiotic use	
Yes / No	37 / 13

irAE: Immune-related adverse event, Grade: Severity of adverse events using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading system, ECOG PS: European cooperative oncology group performance status, ICI: Immune checkpoint inhibitor, IPI: Ipilimumab, Chemo: Chemotherapy

Table 2-1: Univariate analysis of patient background when divided into two groups: irAE Grade 2 or lower and irAE Grade 3 or higher

	irAE Grade under 2 (n=37)	irAE Grade over 3 (n=13)	P-value
Age (years) median (range)	74 (37-89)	64 (37-77)	0.027[a*]
Gender			0.248[b]
Male	27	12	
Female	10	1	
ECOG PS			0.384[c]
0	28	9	
1	7	2	
2	1	2	
3	1	0	
Clinical Stage			0.342[c]
	9	1	
	18	9	
Postoperative relapse	10	3	
Types of ICI			0.035[c*]
ICI	27	4	
ICI+IPI	4	5	
ICI+Chemo	18	5	
ICI+IPI+Chemo	2	2	
Metastatic organs			0.194[c]
0	21	3	
1	12	7	
2	2	2	
3	2	1	
Treatment line			0.398[c]
1st	17	9	
2nd	10	2	
3rd	4	0	
Greater	6	2	

irAE: Immune-related adverse event, Grade: Severity of adverse events using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading system, ECOG PS: European cooperative oncology group performance status, ICI: Immune checkpoint inhibitor, IPI: Ipilimumab, Chemo: Chemotherapy
a Mann-Whitney U test, b Fisher’s exact test, c Chi-squared test
*Statistically significant (P-value < 0.05)

Table 2-2: Univariate analysis of pre-existing blood examinations when divided into two groups: irAE Grade 2 or lower and irAE Grade 3 or higher

	irAE Grade under 2 (n=37)	irAE Grade over 3 (n=13)	P-value
LDH (IU/L) median (range)	211 (98-2625)	214 (167-500)	0.536[a]
ALB (g/L) median (range)	38 (3.5-46)	30 (25-43)	0.132[a]
Cre (mg/dL) median (range)	0.8 (0.5-2.5)	0.9 (0.4-13.6)	0.666[a]
eGFR (mL/min) median (range)	64.3 (24.8-128.8)	68.1 (3.3-182.5)	0.715[a]
AST (IU/L) median (range)	24 (9-160)	30 (17-79)	0.191[a]
ALT (IU/L) median (range)	16 (5-169)	35 (12-119)	0.051[a]
BGL (mg/dL) median (range)	122 (74-194)	110 (89-150)	0.353[a]
HbA1c (%) median (range)	5.7 (4.4-7.7)	5.8 (4.6-7.4)	0.873[a]
WBC (10^3/μL) median (range)	7.0 (2.8-42.6)	8.6 (2.9-20.1)	0.135[a]
Neutrophil (/μL) median (range)	4575 (1110-40035)	6810 (1420-18770)	0.134[a]
Lymphocyte (/μL) median (range)	1355 (350-6060)	1380 (390-2570)	0.803[a]
Platelet (10^3/μL) median (range)	243 (59-764)	350 (14.6-871)	0.472[a]
CRP (mg/dL) median (range)	0.7 (0.1-18.4)	3.7 (0.1-22.5)	0.008[a**]
CK (U/L) median (range)	51 (4-190)	33.5 (13-118)	0.294[a]
FT3 (pg/mL) median (range)	2.7 (1.6-3.5)	2.8 (0.9-4.3)	0.894[a]
FT4 (ng/mL) median (range)	1.2 (1.0-1.6)	1.3 (1.0-1.6)	0.478[a]
TSH (μIU/mL) median (range)	1.8 (0.2-8.8)	1.1 (0.0-3.2)	0.066[a]
ACTH (pg/mL) median (range)	29.2 (1.5-73.7)	29.2 (7.8-75.4)	0.828[a]

irAE, mmune-related adverse event, Grade: the severity of adverse events using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading system ,LDH: lactate dehydrogenase, ALB: albumin, Cre:creatinine, eGFR: estimated glomerular filtration rate, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BGL: blood glucose level, HbA1c: glycated hemoglobin a1c, WBC: white blood cell, CRP: c-reactive protein, CK:creatine kinase, FT3: free triiodothyronine, FT4: free thyroxine, TSH: thyroid stimulating hormone, ACTH: adrenocorticotropic hormone
a Mann-Whitney U test
[**]Statistically significant (P-value < 0.01)

backgrounds, as well as laboratory values of patients who received ICI treatment and subsequently experienced irAEs. By examining these factors, we seek to gain valuable insights into potential risk factors contributing to the occurrence of irAEs and advance our understanding of this aspect of ICI treatment.

2. Investigations and results

2.1. Patient background

Fifty eligible patients were assessed for the severity of adverse events using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading system. The patient background is presented in Table 1.

In the subsequent analysis, patients were separated into two groups based on the severity of irAEs. The first group comprised patients with irAE Grade 2 or lower (referred to as irAE Grade under 2), while the second group consisted of patients with irAE Grade 3 or higher (referred to as irAE Grade over 3). Notably, statistically significant differences were observed in age (p=0.027) and C-reactive protein (CRP) levels (p=0.008) between these two groups, as shown in Table 2-1 and Table 2-2.

The ICI regimens employed in the study were classified into four groups: ICI alone (ICI), combination of ICI with the same agent (ICI+IPI: Ipilimumab), combination of ICI with an anticancer agent (ICI+Chemo), and combination of ICI with the same agent and an anticancer agent (ICI+IPI+Chemo). Upon analyzing the two groups of patients, one with irAE Grade under 2 and the other with irAE Grade over 3, a statistically significant difference was observed (p=0.035), as shown in Table 2-1. Additionally, from the cross-tabulation table, a chi-square value of 8.582, a phi coefficient of 0.358, and Cramer's V value of 0.358 were obtained.

2.2. Multivariate Analysis

In the multivariate analysis, the contribution of each individual factor as a risk factor for the occurrence and severity of irAEs was evaluated. The factors considered were age and CRP, as they had shown statistically significant differences in the univariate analysis of patient background. The forced entry method yielded results for age (95% CI: 0.928-1.042, P=0.387) and CRP (95% CI: 0.998-1.374, P=0.063).

2.3. ROC (Receiver Operating Characteristic) Analysis

ROC analysis was conducted to assess the predictive performance of the model using age and CRP, considering their statistically significant differences in the univariate analysis of patient background.

The cutoff value for age was 73.0 (sensitivity 0.514, specificity 0.923, accuracy 0.56) (Figure 1A). The cutoff value for CRP was 1.465 (sensitivity 0.846, specificity 0.730, accuracy 0.58) (Figure 1B).

The incidence of irAE Grade 3 or higher was higher in the patients under 73 years old compared with those 73 years old or older (P=0.008) (Figure 2A). The incidence of irAE Grade 3 or higher was higher in the CRP 1.465 or higher group (P<0.001) (Figure 2B). There was no difference in the incidence of irAE Grade 3 or higher in the two groups (Figure 2C).

2.4. Correlation between exposure and irAE Grade

In our investigation, we directed our attention to the relationship between exposure and the frequency of irAEs, as depicted in Fig.

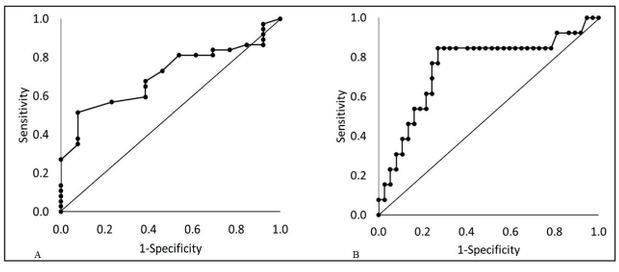

Fig. 1: ROC analysis was conducted to assess the predictive performance of the model using age and CRP. (A) ROC curve of age, (B) ROC curve of CRP. ROC: Receiver Operating Characteristic, CRP: C-reactive protein

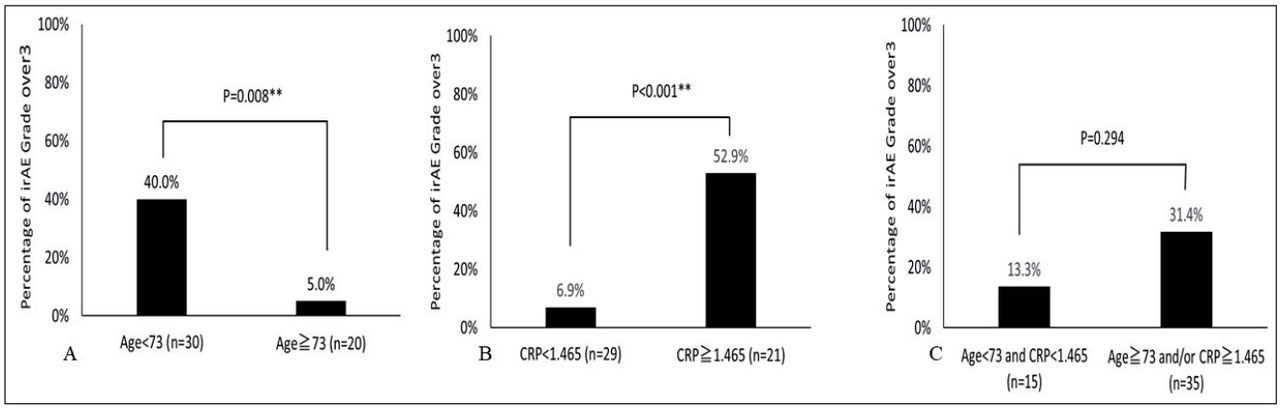

Fig. 2: Comparison of the incidence of irAE Grade 3 or higher (A) in the age cutoff of less than 73 years and 73 years or older groups, (B) in the CRP cutoff of less than 1.465 and 1.465 or higher groups, (C) in the age <73 years and CRP <1.465 vs. age >73 years and/or CRP >1.465 groups. The p-value was calculated using Fisher's exact test.
irAE: Immune-related adverse event, Grade: Severity of adverse events using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading system, CRP: C-reactive protein
**Statistically significant (P-value < 0.01)

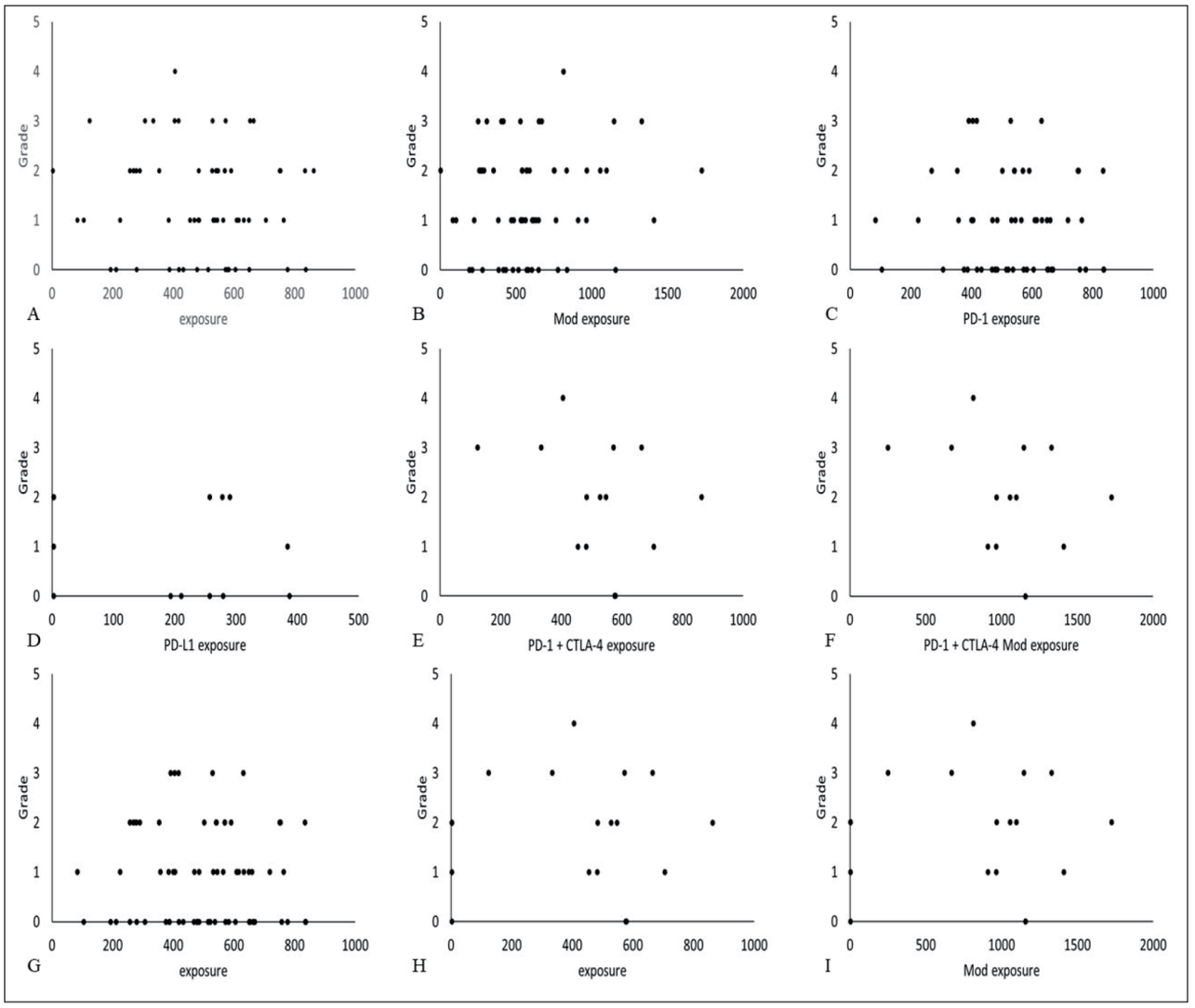

Fig. 3: Relationship between exposure and the frequency of irAEs.
(A) exposure vs irAE Grade, (B) Mod exposure vs irAE Grade, (C) PD-1 exposure vs irAE Grade, (D) PD-L1 exposure vs irAE Grade, (E) PD-1 + CTLA-4 exposure vs irAE Grade, (F) PD-1 + CTLA-4 Mod exposure vs irAE Grade, (G) Exposure group with fixed dose of ICI vs irAE Grade, (H) Exposure group with ICI administered at a dose per body weight vs irAE Grade, (I) Mod exposure group with ICI administered at a dose per body weight vs irAE Grade
irAE: Immune-related adverse event, ICI: Immune checkpoint inhibitor, Grade: Severity of adverse events using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading system, PD-1: Programmed death receptor-1, PD-L1: Programmed cell death ligand1, CTLA-4: Cytotoxic T-lymphocyte associated antigen-4

3A. Exposure was defined in Eq. (1). However, despite analyzing these factors, no correlation was found between exposure and the severity of irAEs (irAE Grade).

Continuing our investigation, we specifically examined the ipilimumab (IPI) combination group, in which the steady-state mean blood concentration of IPI (Cssave) was relatively low. Despite doubling the exposure by combining IPI with other immune checkpoint inhibitors (Mod exposure), no correlation was observed between this increased exposure and irAE Grade (Fig. 3B).

In this analysis, exposure to ICIs was categorized into three groups: the PD-1 group, PD-L1 group, and PD-1+CTLA-4 group. Across all these groups, there was no observed correlation between exposure levels, including both set exposure and mod exposure, and irAE Grade (Figs. 3C to 3F).

In the subsequent analysis, exposure and mod exposure were further classified into two groups based on administration methods: the fixed-dose ICI group and the dose-per-weight ICI group. Despite this division, no correlation was observed between exposure, mod exposure, and irAE Grade in either of these groups (Figs. 3G to 3I).

3. Discussion

In this study, when the patients were categorized into two groups based on irAE Grade, specifically irAE Grade under 2 and irAE Grade over 3, statistically significant differences were observed in age and CRP levels among the background factors between the two groups.

The findings of this study regarding the higher incidence of Grade 3 or higher irAEs in the younger age group contrast with the existing reports, which suggest that being under 60 years of age is a risk factor for ICI-related pneumonia (Asada et al. 2021). In this study, the cutoff age for higher incidence was determined to be 73 years, which deviates from the age reported in previous studies. Several factors may contribute to this discrepancy. First, the longer life expectancy in Japan compared to other countries might influence the age-related trends in irAE occurrence. Additionally, this study examined the incidence of irAEs other than pneumonia, which could contribute to different findings. Moreover, focusing on Grade 3 or higher irAEs, which are considered severe and require more attention and treatment, may contribute to distinct patterns in age-related risk.

The reported association between a rapid rise in CRP, independent of infections, as a biomarker to predict irAEs, adds to the relevance of CRP as a potential indicator of irAE development (Abolhassani et al. 2019). In this study, while the variation of CRP was not confirmed, it was found that the rate of Grade 3 or higher irAE occurrence was higher in the group with higher CRP values before ICI administration. CRP is synthesized in the liver and increases when inflammation or tissue damage occurs. The occurrence of irAEs can lead to inflammation reactions and tissue damage, which may result in an elevation of CRP. Additionally, when irAEs cause more severe symptoms, it is possible that the associated inflammation reactions are intensified, further contributing to an increase in CRP levels.

The cutoff value for CRP obtained in the ROC analysis was 1.465. Since the reference range for CRP is typically 0.3 mg/dL or lower, it suggests that there may have been some inflammation or tissue damage present before ICI administration. Consequently, this pre-existing condition might have contributed to the development of severe irAEs as an outcome.

This observation emphasizes the potential utility of CRP as a readily available and routinely measured biomarker in predicting the risk of severe irAEs.

Furthermore, while statistically significant differences were observed among different ICI treatment groups in the occurrence of irAEs, judging from the values of the phi coefficient and Cramer's V, it can be considered a relatively weak correlation.

The endpoint of the case report form (CRF) for this study was established to capture the occurrence and severity of irAEs during the period of ICI administration. However, it is essential to recognize that irAEs can continue to occur even after the completion of ICI treatment. The duration of observation for irAEs in the future depends on several factors, including the specific ICI being used, the patient's individual characteristics, and the duration of immune system alterations caused by ICI treatment. Some irAEs may emerge days or weeks after ICI administration is completed, while others could develop months or even years later. Given this potential for delayed onset irAEs, it is crucial to maintain long-term follow-up and monitoring of patients even after the completion of ICI treatment. Healthcare providers should remain vigilant for any signs of irAEs during regular follow-up appointments, and patients should be educated about the possibility of late-onset irAEs so that they can report any symptoms promptly. Close and continued monitoring will allow for timely detection and management of any late-emerging irAEs, contributing to improved patient care and safety.

Indeed, several studies have reported intriguing associations between the development of irAEs and better prognosis in terms of overall survival and progression-free survival for patients undergoing ICI treatment (Haratani et al. 2018). Additionally, there are reports suggesting that patients who develop irAEs within two weeks of starting ICI treatment may have better progression-free survival rates (Teraoka et al. 2017). The "early onset irAE" hypothesis proposes that early occurrence of irAEs might reflect a more robust and effective immune response against the tumor, leading to improved treatment outcomes. While these observations are intriguing, it is crucial to note that not all studies have consistently shown these associations, and the underlying mechanisms behind these phenomena require further investigation. Nevertheless, identifying accurate monitoring parameters, including potential correlations with the occurrence and timing of irAEs, may indeed be relevant to understanding the long-term prognosis and treatment outcomes of patients using ICIs. Such insights could inform the development of personalized treatment approaches and improve patient management strategies in the context of immunotherapy.

In the current study, patients with pre-existing autoimmune diseases who were already receiving continuous systemic treatment with corticosteroids or immunosuppressive drugs were excluded from the analysis. The exclusion of such patients is a common practice in studies involving ICI treatment to focus on a more homogeneous patient population and to better understand the specific effects of ICIs on the immune system.

Indeed, administering ICIs to patients with pre-existing autoimmune diseases can lead to increased production of autoantibodies due to B cell activation, potentially exacerbating the underlying autoimmune condition (Ramos-Casals et al. 2020). This phenomenon can result in worsening symptoms and increased disease activity, making it challenging to manage both the autoimmune disease and the cancer simultaneously. Moreover, reports suggest that patients with autoimmune diseases may be more likely to discontinue anti-PD-1 therapy due to treatment-related toxicity. Additionally, patients with inflammatory bowel disease (IBD) are at a higher risk of developing colitis induced by anti-PD-1 therapy (van der Kooij et al. 2021). While excluding patients with autoimmune diseases undergoing continuous immunosuppressive treatment in the present study may have provided a clearer understanding of the relationship between ICIs and irAEs, it is essential to acknowledge the need for further data collection that includes patients with autoimmune diseases. Comprehensive studies involving these patients can provide valuable insights into the safety, efficacy, and management of ICIs in this specific patient population.

It's essential to emphasize that in this study, no correlation was found between irAEs and the exposure set, indicating that the occurrence of irAEs was not significantly related to the level of drug exposure. Indeed, the reported incidence of severe adverse events, including irAEs, associated with anti-PD-1/PD-L1 antibodies has shown to be less dose-dependent in previous studies (Kumar et al. 2017). This finding suggests that increasing the dosage of anti-PD-1/PD-L1 antibodies may not necessarily result in a proportional increase in the severity of adverse events. Future studies should continue to explore and understand the factors influ-

encing the occurrence and severity of irAEs, including the role of drug exposure.

Limitations of this study include that it is a non-randomized, backward-looking study, which may have significant bias in patient selection and information collection. In addition, the study was conducted at a single institution, which may have resulted in patient bias. In addition, the results of the analysis were based mainly on laboratory values immediately before administration, and did not take into account the onset of irAE or fluctuations in values. Further investigation is needed to increase the number of patients in a multicenter study.

The results of this study suggest that age and CRP levels are related to the occurrence of irAEs of Grade 3 or higher. Specifically, the study found statistically significant differences in age and CRP levels between the two groups of patients with irAE Grade under 2 and irAE Grade over 3.

4. Experimental

4.1. Subject of the survey

Patients who received ICIs (including single agents and combinations) or ICIs in combination with anticancer agents at our hospital between April 2020 and May 2022 were included in the study. Patients with autoimmune diseases who were receiving continuous systemic treatment with corticosteroids or immunosuppressive drugs were excluded.

4.2. Survey items

The survey items included age, gender, height, weight, performance status, clinical stage, cancer type, types of ICI, number of transplant organs, treatment line, smoking status, history of steroid use, history of antibiotic use, LDH, ALB, Cre, eGFR, AST, ALT, BGL, HbA1c, WBC, Neutrophil, Lymphocyte, Platelet, CRP, CK, FT3, FT4, TSH, ACTH.

4.3. Adverse event evaluation methods

Grades were evaluated according to CTCAE (Common Terminology Criteria for Adverse Events) v5.0. Grade 3 or higher was defined as serious adverse drug reactions, and Grades 1 and 2 were also analyzed and evaluated as risk factors.

4.4. CRF (Case Report From)

A case report form (CRF) was prepared for the study and defined as follows One CRF sheet was defined as a case report of one treatment of a patient in which ICIs were administered. One course is defined as one unit of ICIs administered according to a regimen (including concomitant medications and withdrawal). One treatment was defined as the duration of drug administration to the treatment endpoint by multiple courses (including only one course) of the same regimen. A treatment endpoint was defined as a temporary termination of the regimen due to withdrawal, discontinuation, or termination of treatment for more than 3 days outside the regimen. One CRF was defined as 10 courses of treatment.

4.5. Exposure

In this study, exposure was defined as eq1, similar to Shulgin et al. IC50 is a measure of the ability of a drug to inhibit the binding of a ligand to its target, calculated from in vitro data of human targets and molecular weight. IC50 and molecular weight for each compound are shown below.

The factors influencing clearance were determined based on literature references, and calculated from individual patient parameters obtained using CRF. Next, the focus was on the group receiving IPI. The steady-state mean blood concentration of IPI, defined as Cssave, was relatively low, and when exposure was doubled by combining it with IPI, this was designated as Mod exposure.

The study also investigated the correlation between Mod exposure and the incidence rate of irAEs.

exposure = Cssave/IC50 Eq. (1) (Shulgin et al.2020)

Cssave: mean steady-state blood concentration (mg/L), IC50: 50% inhibitory concentration (mg/L)

4.6. Statistical analysis

Chi-square test or Fisher's exact test will be used to evaluate the association between individual factors and the occurrence of irAE in each patient. Multiple regression analysis will be used to analyze and evaluate the contribution of individual factors as risk factors for each patient's multiple individual factors, irAE expression, and grade. We will also assess the predictive capability of the model using Receiver Operating Characteristic curves. The data analysis was conducted using IBM SPSS Statistics Ver 29.0.

4.7. Ethical considerations

This study was conducted in accordance with the "Ethical Guidelines for Medical Research Involving Human Subjects" and was approved by the Ethical Review Committee of the International University of Health and Welfare (approval number: 21-Ig-163-2).

Conflict of interest: None declared.

References

Abolhassani A-R, Schuler G, Kirchberger MC, Heinzerling L (2019) C-Reactive Protein as an Early Marker of Immune-Related Adverse Events. J Cancer Res Clin Oncol 145: 2625–2631.

Asada M, Mikami T, Niimura T, Zamami Y, Uesawa Y, Chuma M, Ishizawa K (2021) The risk factors associated with immune checkpoint inhibitor-related pneumonitis. Oncology 99: 256–259.

Haratani K, Hayashi H, Chiba Y, Kudo K, Yonesaka K, Kato R, Kaneda H, Hasegawa Y, Tanaka K, Takeda M, Nakagawa K (2018) Association of immune-related adverse events with nivolumab efficacy in non-small-cell lung cancer. JAMA Oncol 4: 374–378.

Kumar V, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB (2017) Current diagnosis and management of immune-related adverse events (irAEs) induced by immune checkpoint inhibitor therapy. Front Pharmacol 8: 49.

Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS, Wolchok JD (2015) Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 373: 23–34.

Okada N, Matsuoka R, Sakurada T, Goda M, Chuma M, Yagi K, Zamami Y, Nishioka Y, Ishizawa K (2020) Risk factors of immune checkpoint inhibitor-related interstitial lung disease in patients with lung cancer: a single-institution retrospective study. Sci Rep 10: 13773.

Postow MA, Sidlow R, Hellmann MD (2018) Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med 378: 158–168.

Ramos-Casals M, Brahmer JR, Callahan MK, Flores-Chávez A, Keegan N, Khamashta MA, Lambotte O, Mariette X, Prat A, Suárez-Almazor ME (2020) Immune-related adverse events of checkpoint inhibitors. Nat Rev Dis Primers 6: 38.

Shulgin B, Kosinsky Y, Omelchenko A, Chu L, Mugundu G, Aksenov S, Pimentel R, DeYulia G, Kim G, Peskov K, Helmlinger G (2020) Dose dependence of treatment-related adverse events for immune checkpoint inhibitor therapies: a model-based meta-analysis. Oncoimmunology 9: 1748982.

Teraoka S, Fujimoto D, Morimoto T, Kawachi H, Ito M, Sato Y, Nagata K, Nakagawa A, Otsuka K, Uehara K, Imai Y, Ishida K, Fukuoka J, Tomii K (2017) Early immune-related adverse events and association with outcome in advanced non-small cell lung cancer patients treated with nivolumab: a prospective cohort study. J Thorac Oncol 12: 1798–1805.

van der Kooij MK, Suijkerbuijk KPM, Aarts MJB, van den Berkmortel FWPJ, Blank CU, Boers-Sonderen MJ, van Breeschoten J, van den Eertwegh AJM, de Groot JWB, Haanen JBAG, Hospers GAP, Piersma D, van Rijn RS, Ten Tije AJ, van der Veldt AAM, Vreugdenhil G, van Zeijl MCT, Wouters MWJM, Dekkers OM, Kapiteijn E (2021) Safety and efficacy of checkpoint inhibition in patients with melanoma and preexisting autoimmune disease: a cohort study. Ann Intern Med 174: 641–648.

Drug	Target	IC50	Molecular weight	IC50 (mg/L)
Nivolumab	PD-1	1.04 (nmol/L)	145000	0.1508
Pembrolizumab	PD-1	625 (pM)	149000	0.093125
Durvalumab	PD-L1	0.10 (nM)	149000	0.0149
Atezolizumab	PD-L1	82.8±40.3 (pmol/L)	144610.6	0.011973754
Avelumab	PD-L1	0.071 (nM)	147000	0.010437
Ipilimumab	CTLA-4	3.42 (μg/mL)	148000	3.42