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Original Article

Comparison of immune-related adverse events and analysis of risk factors in older and younger rectal cancer patients receiving immunotherapy

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Abstract: Background: Immunotherapy has transformed rectal cancer treatment but poses risks of immune-related adverse events (irAEs), particularly in elderly patients who exhibit immunosenescence and inflammaging. This study compares the incidence and severity of irAEs in elderly and young rectal cancer patients receiving immunotherapy and identifies predictive biomarkers for these events. Methods: We retrospectively analyzed 405 rectal cancer patients treated with immunotherapy from January 2015 to December 2023. Patients were categorized into younger (< 60 years) and older (≥ 60 years) groups. Incidence and severity of irAEs were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) standards. Blood samples were analyzed for hematological and immunological markers. Results: The older group displayed a significantly higher incidence of irAEs at 48.65% compared to 32.11% in the younger group ($P = 0.003$). Severity varied, with 69.72% of younger patients experiencing irAEs of grade ≤ 2 versus 51.69% in the older group ($P = 0.001$). Notably, higher absolute lymphocyte count (ALC), interleukin-6 (IL-6), and C-reactive protein (CRP) levels were associated with increased irAEs ($P = 0.002$, $P = 0.001$, $P = 0.007$, respectively). The multivariate analysis identified ALC, IL-6, CRP, B and T Lymphocyte Attenuator, Human Granulocyte-macrophage Colony Stimulating Factor, Programmed Death-1 and Programmed Death-Ligand 1 as significant predictors of irAEs, with ALC showing an odds ratio (OR) of 9.700 ($P = 0.001$) and IL-6 an OR of 58.961 ($P < 0.001$). Furthermore, the platelet-to-lymphocyte ratio (PLR) inversely correlated with irAEs ($P = 0.013$). Conclusion: Older rectal cancer patients receiving immunotherapy were at increased risk for both greater incidence and severity of irAEs. Specific biomarkers, such as ALC and IL-6, were associated with a heightened risk of these events.

Keywords: Rectal cancer, immunotherapy, immune-related adverse events, age differences, biomarkers, hematologic predictors

Introduction

The advent of immunotherapy has revolutionized cancer treatment, offering promising therapeutic avenues for various malignancies, including rectal cancer [1, 2]. The utilization of immune checkpoint inhibitors, such as those targeting Programmed Death-1 (PD-1) and Programmed Death-Ligand 1 (PD-L1), has particularly garnered attention owing to their capacity to reinvigorate antitumor immune responses [3, 4]. However, this newfound therapeutic landscape was not devoid of challenges, particularly concerning immune-related adverse events (irAEs), which present a unique spectrum of side effects distinct from conventional chemotherapy [5, 6]. These adverse events were attributable to the nonspecific activation of the immune system, potentially leading to self-reactivity and autoimmunity [7].

While immunotherapy has shown remarkable success in the treatment of malignancies such as melanoma and non-small cell lung cancer, its application in rectal cancer remains nuanced. Notably, only a subset of rectal cancer patients-particularly those with mismatch repair-deficient (dMMR) or microsatellite instability-high (MSI-H) tumors-demonstrate sustained responses to immune checkpoint inhibitors (ICIs) [8]. This heterogeneity underscores the need to optimize patient selection and manage treatment-related risks, especially irAEs.

The incidence and severity of irAEs were notably variable among patients undergoing immunotherapy, often influenced by demographic factors such as age [9]. The aging immune system, characterized by phenomena such as immunosenescence and inflammaging, presents distinct immunological challenges [10]. Immunosenescence describes the progressive decline in immune function that occurs as part of the natural aging process, leading to weakened adaptive immune responses [11, 12]. In contrast, inflammaging is a condition commonly observed in older individuals, marked by persistent, low-level inflammation and increased concentrations of pro-inflammatory cytokines [13, 14]. These age-related changes have profound implications for the use of immunotherapy in older adults, potentially altering their response and resilience to treatment-induced immune activation.

Given the increasing prevalence of rectal cancer among the aging population, it was paramount to comprehend how age influences the occurrence and nature of irAEs in rectal cancer patients. Although immunotherapy offers considerable promise in extending survival for rectal cancer patients, the risk of irAEs necessitates a nuanced understanding to optimize treatment regimens, particularly for elderly patients who may be more susceptible to these adverse outcomes. Younger patients, on the other hand, typically exhibit a more robust immune response, which may influence both the therapeutic efficacy and the risk profile of immunotherapy.

However, the anatomical and immunological uniqueness of the rectal microenvironment - such as its dense commensal microbiota and high baseline mucosal immune activity - predisposes patients to distinct irAE profiles [15]. For instance, colitis is a frequently reported irAE in rectal cancer patients receiving ICIs, likely due to the overlap between antitumor immunity and intestinal homeostasis [16]. Other common irAEs include hepatitis, endocrine disorders, and pneumonitis, which collectively threaten treatment continuity and patient survival [17-19].

Despite the critical need to tailor immunotherapy to address age-specific risks, existing literature lacks a comprehensive analysis comparing irAEs between elderly and young rectal cancer patients. Current guidelines for irAE management in immunotherapy for rectal cancer remain largely age-agnostic. Previous studies have either focused on irAEs in pan-cancer cohorts or neglected to compare age-specific risks in rectal cancer patients [20]. This study seeks to fill this gap by examining not only the incidence and severity of irAEs in a cohort of rectal cancer patients stratified by age but also by identifying potential hematologic and immunologic predictors of these events.

Materials and methods

Study design

A retrospective analysis was performed on 405 rectal cancer patients who underwent immunotherapy at the Second People's Hospital of Yibin between January 2015 and December 2023. The patients were divided based on the occurrence of irAEs. Patients who did not experience irAEs were categorized into the No-irAEs group (n = 243), whereas those who did experience irAEs were placed into the irAEs group (n = 162). Furthermore, the same patient cohort was subdivided into two age groups-younger and older-using the commonly used threshold of 60 years [21]: the younger group comprised individuals under 60 years of age (n = 109), while the older group included those aged 60 years and above (n = 296). This is also the standard used by the World Health Organization to classify individuals as older adults or middle-aged and younger adults [22].

All patients received either anti-PD-1/PD-L1 inhibitors or combined therapies including CTLA-4 inhibitors. The specific regimens included pembrolizumab, nivolumab, and ipilimumab. Notably, the choice of regimen was based on clinical guidelines and patient-specific factors. Potential differences in irAEs due to different drug combinations were considered in our analysis.

The Institutional Review Board and Ethics Committee of the Second People's Hospital of Yibin approved the study. Informed consent was waived as the study was retrospective, using only de-identified patient data, thus posing no risk to patient care. This waiver aligned with regulatory and ethical standards.

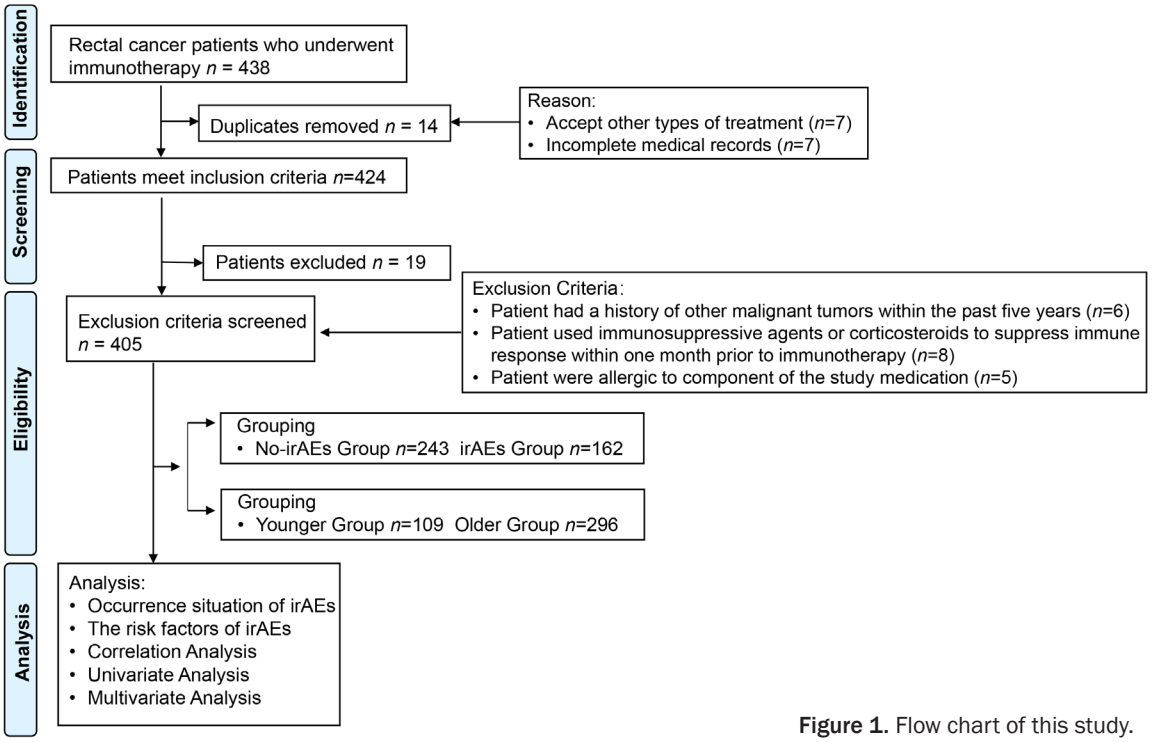

Figure 1. Flow chart of this study.

Inclusion and exclusion criteria

Inclusion criteria: Patients were eligible for inclusion if they were diagnosed with rectal cancer in accordance with established guidelines [23], received immunotherapy, were 18 years of age or older, and had comprehensive medical records free of missing information.

Exclusion criteria: Patients were excluded if they had a history of other malignant tumors within the past five years, used immunosuppressive agents or corticosteroids to suppress immune responses within one month prior to immunotherapy, had infectious diseases, hematological disorders, or autoimmune diseases within two weeks prior to immunotherapy that could influence inflammatory markers, suffered from severe cardiac, hepatic, or renal insufficiency, or were allergic to any component of the study medications. **Figure 1** shows the flowchart of the study.

Data collection

Patient data were extracted from the medical record system and included demographic characteristics, incidence and types of irAEs, blood test results, levels of inflammatory markers in peripheral blood, as well as levels of growth factors and immune regulatory factors. Biological age was determined using an established algorithm that integrates multiple biomarkers, including telomere length, DNA methylation patterns, and functional assessments of immune cells. This metric aims to provide a more accurate reflection of an individual's physiological state compared to chronological age. Biological Age = Actual Age + (Points Added Due to Bad Habits - Points Subtracted Due to Good Habits) × Age Factor.

The Eastern Cooperative Oncology Group (ECOG) performance status was employed to assess patients' overall health condition and their ability to tolerate treatment, using physical activity levels as an indicator [24]. Detailed definitions for each ECOG score are provided in Supplementary Table 1. This scoring system ranges from 0 (fully active) to 5 (deceased), with higher scores indicating poorer performance status. The reliability of the ECOG performance status was indicated by Cohen's κ = 0.486 [25].

The extent of tumor progression in patients was assessed using the tumor-node-metastasis (TNM) staging system [26], an internationally recognized framework for classifying tumor advancement, where higher stages indicate more advanced disease. The system was divided into three components: T (Tumor), N

(Node), and M (Metastasis). Detailed definitions for each component of the TNM staging system are provided in Supplementary Table 2.

After four weeks of immunotherapy, patients underwent imaging examinations with a dual-source CT scanner (SOMATOM Force, Shanghai Siemens Medical Instruments Co., Ltd., China) to assess the effectiveness of the treatment. The evaluation followed the Response Evaluation Criteria in Solid Tumours version 1.1 guidelines [27]:

Complete Response (CR): Complete disappearance of all measurable tumor lesions for at least four weeks. Partial Response (PR): A minimum 30% reduction in the combined longest diameters of target lesions from baseline measurements, with no new lesions developing. Stable Disease (SD): Alterations in the combined longest diameters of target lesions do not surpass a 20% increase or a 30% reduction from baseline, and no new lesions are detected. Progressive Disease (PD): Over a 20% increase in the combined longest diameters of target lesions compared to the last assessment, with an absolute increase of at least 5 millimeters, or the emergence of one or more new lesions.

irAEs

Instances of irAEs were assessed in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) guidelines [28]. The CTCAE uses a five-grade scale to assess the severity of adverse events.

Grade 1: Mild; no intervention required. Grade 2: Moderate; may necessitate minor, noninvasive treatment. Grade 3: Severe; significant symptoms or signs; medical care needed; interferes with daily activities. Grade 4: Life-threatening; requires urgent intervention. Grade 5: Death.

Higher grades correspond to severer adverse events. The intra-class correlation coefficients (ICC) were found to be ≥ 0.70, indicating good reliability [29].

Blood test

Within 24 hours of admission, 5 mL of venous and arterial blood was collected from fasting patients. Plasma was separated using a low-temperature high-speed centrifuge and stored at -80°C for subsequent analysis. Complete blood counts (including white blood cell count, absolute lymphocyte count, hemoglobin, and albumin) were performed using an automated hematology analyzer. The monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR) were derived from these hematological parameters.

Serum levels of inflammatory cytokines [Interleukin-6 (IL-6), Interleukin-22 (IL-22), C-Reactive Protein (CRP), Procalcitonin (PCT)], immune checkpoint molecules [Programmed Death-1 (PD-1), Programmed Death-Ligand 1 (PD-L1), B and T Lymphocyte Attenuator (BTLA)], and hematopoietic factors [Stem Cell Factor (SCF), Granulocyte-macrophage Colony Stimulating Factor (GM-CSF)] were quantified using an automated biochemistry analyzer and enzyme-linked immunosorbent assay (ELISA), respectively. Detailed protocols for sample processing, instrumentation specifications, and assay methodologies are provided in Supplementary Table 3.

Statistical analysis

SPSS 29.0 was used for data analysis. Categorical data were presented as counts and percentages [n (%)] and chi-square test (χ^2) was used. Continuous data were checked for normality using the Shapiro-Wilk test. Normally distributed data were expressed as mean ± standard deviation ($X \pm s$) using t test with corrected variance, while non-normally distributed data were reported as median with interquartile range [median (25th, 75th percentiles)] using the Wilcoxon rank-sum test. Statistical significance was set at $P < 0.05$. Correlation analysis utilized Pearson's method for continuous variables and Spearman's method for categorical variables. Univariate and multivariate analyses were also conducted to assess factors influencing irAEs. Univariate analysis included factors such as Absolute Lymphocyte Count (ALC), albumin (ALB), PLR, IL-6, IL-22, CRP, SCF, BTLA, GM-CSF, PD-1, and PD-L1. These factors were selected based on prior literature and their potential impact on immune responses and strong association with irAEs.

Table 1. Comparison of demographic characteristics between the No-irAEs group and the irAEs group

	No-irAEs group (n = 233)	irAEs group (n = 172)	t/χ^2	P
Age (years)	54.65 ± 6.12	68.76 ± 5.45	24.029	< 0.001
Biological Age (years)	54.28 ± 5.18	68.62 ± 5.32	27.215	< 0.001
Female/Male	102 (43.78%)/131 (56.22%)	82 (47.67%)/90 (52.33%)	0.606	0.436
Ethnicity (Han/Other)	184 (78.97%)/49 (21.03%)	137 (79.65%)/35 (20.35%)	0.028	0.867
BMI (kg/m^2)	23.48 ± 2.49	23.75 ± 2.61	1.085	0.279
ECOG performance status (0/≥ 1)	185 (79.4%)/48 (20.6%)	137 (79.65%)/35 (20.35%)	0.004	0.950
Smoking history (Yes/No)	86 (36.91%)	58 (33.72%)	0.439	0.508
Drinking history (Yes/No)	58 (24.89%)	46 (26.74%)	0.178	0.673
Hypertension (Yes/No)	79 (33.91%)	60 (34.88%)	0.042	0.838
Diabetes (Yes/No)	83 (35.62%)	55 (31.98%)	0.585	0.444
Educational level (high school or below/college or above)	32 (13.73%)/201 (86.27%)	25 (14.53%)/147 (85.47%)	0.052	0.819
Marital Status (Married/Unmarried)	193 (82.83%)/40 (17.17%)	147 (85.47%)/25 (14.53%)	0.509	0.476
RIP (< 1/1-3/3)	58 (24.89%)/97 (41.63%)/78 (33.48%)	48 (27.91%)/82 (47.67%)/42 (24.42%)	3.901	0.142
TNM stage (≤ II/> II)	111 (47.64%)/122 (52.36%)	73 (42.44%)/99 (57.56%)	1.078	0.299
Distance from anal verge (≤ 5/> 5)	62 (26.61%)/171 (73.39%)	41 (23.84%)/131 (76.16%)	0.401	0.527
Tumour size (cm)	3.41 ± 1.04	3.53 ± 1.01	1.125	0.261

BMI: Body Mass Index; ECOG: Eastern Cooperative Oncology Group performance status; RIP: the ratio of family income to poverty; TNM: tumor node metastasis classification.

Table 2. Multivariate analysis of age in the occurrence of irAEs

	Coefficient	Stand Error	Wald	P	OR	OR CI Lower	OR CI Upper
Age (years)	0.495	0.094	5.280	< 0.001	1.640	1.365	1.971
Biological Age (years)	0.571	0.098	5.810	< 0.001	1.769	1.459	2.145

OR: Odds Ratio; OR CI Lower: Odds Ratio Confidence Interval Lower Bound; OR CI Upper: Odds Ratio Confidence Interval Upper Bound.

Results

Age and immune-related adverse events

In the comparison of demographic characteristics between the No-irAEs group (n = 233) and the irAEs group (n = 172), significant differences were observed in age and biological age. The irAEs group had significantly higher mean age (68.76 ± 5.45 years) compared to the No-irAEs group (54.65 ± 6.12 years) (t = 24.029, P < 0.001) (**Table 1**). Similarly, the biological age was also significantly higher in the irAEs group (68.62 ± 5.32 years) compared to the No-irAEs group (54.28 ± 5.18 years) (t = 27.215, P < 0.001). No significant differences were observed between the No-irAEs and irAEs groups in terms of gender distribution, ethnicity, body mass index, ECOG performance status, smoking history, drinking history, hypertension prevalence, diabetes prevalence, educational level, marital status, the ratio of family income to poverty (RIP) categories, TNM stage, distance from anal verge, or tumor size (P > 0.05).

In the multivariate analysis of factors related to irAEs, both age and biological age were identified as independent risk factors for the occurrence of irAEs (**Table 2**). Specifically, the odds ratio (OR) for age was 1.640 (95% CI: 1.365 to 1.971), with a coefficient of 0.495, standard error of 0.094, Wald statistic of 5.280, and P < 0.001. Similarly, the OR for biological age was 1.769 (95% CI: 1.459 to 2.145), with a coefficient of 0.571, standard error of 0.098, Wald statistic of 5.810, and P < 0.001.

Basic data comparison

The mean age differed substantially between the two groups, with the younger cohort having an average age of 43.63 years (SD = 8.54) compared to 73.66 years (SD = 7.57) for the older group (t = 34.193, P < 0.001) (**Table 3**). And the biological age between the two groups is 44.25 ± 8.66 and 74.21 ± 8.27 (t = 34.193, P < 0.001). Notably, a significant discrepancy was observed in the ECOG performance status between the two groups, wherein 85.32% of younger patients had a status of 0, as opposed to 73.31% of older patients having an ECOG performance status of ≥ 1 (χ^2 = 6.400, P = 0.011). Furthermore, a notable variance was observed in the distributions of TNM stage among patients in the younder and older groups, as a higher percentage of younger patients were at stage II or below compared to older patients (51.38% versus 39.19%, χ^2 = 4.843, P = 0.028). Other variables showed no statistically significant differences between the two age groups (all P > 0.05). These findings underscore the importance of assessing age-specific characteristics in the management of elderly and young rectal cancer patients undergoing immunotherapy.

Therapeutic effects

In the comparison of the curative effect between the younger and older groups, several trends were observed in treatment responses, although the overall difference did not reach statistical significance (χ^2 = 7.756, P = 0.051) (**Table 4**). Specifically, the younger group had a higher proportion of CR cases (6.42%, n = 7) compared to the Older group (2.03%, n = 6). The Younger group also showed a slightly higher proportion of PR (50.46%, n = 55) compared to the Older group (43.92%, n = 130). A higher proportion of patients in the Older group had SD (17.57%, n = 52) compared to the Younger group (11.93%, n = 13). Similarly, the Older group had a higher proportion of PD (36.49%, n = 108) compared to the Younger group (31.19%, n = 34).

Immune-related adverse events occurrence

The younger cohort experienced a lower incidence rate of 28.44%, compared to 44.26% in the older group (χ^2 = 8.304, P = 0.004) (**Table 5**). Furthermore, the severity of irAEs, as mea-

Table 3. Comparison of demographic characteristics between the younger group and the older group

	Younger group (n = 109)	Older group (n = 296)	t/χ^2	P
Age (years)	43.63 ± 8.54	73.66 ± 7.57	34.193	< 0.001
Biological Age (years)	44.25 ± 8.66	74.21 ± 8.27	31.916	< 0.001
Female/Male	50 (45.87%)/59 (54.13%)	142 (47.97%)/154 (52.03%)	0.141	0.707
Ethnicity (Han/Other)	89 (81.65%)/20 (18.35%)	233 (78.72%)/63 (21.28%)	0.421	0.516
BMI (kg/m^2)	23.64 ± 2.55	24.24 ± 2.84	1.939	0.053
ECOG performance status (0/≥ 1)	93 (85.32%)/16 (14.68%)	217 (73.31%)/79 (26.69%)	6.400	0.011
Smoking history (Yes/No)	40 (36.7%)	106 (35.81%)	0.027	0.869
Drinking history (Yes/No)	27 (24.77%)	85 (28.72%)	0.620	0.431
Hypertension (Yes/No)	37 (33.94%)	109 (36.82%)	0.286	0.592
Diabetes (Yes/No)	39 (35.78%)	100 (33.78%)	0.141	0.707
Educational level (high school or below/college or above)	15 (13.76%)/94 (86.24%)	50 (16.89%)/246 (83.11%)	0.579	0.447
Marital Status (Married/Unmarried)	89 (81.65%)/20 (18.35%)	262 (88.51%)/34 (11.49%)	3.246	0.072
RIP (< 1/1-3/3)	28 (25.69%)/45 (41.28%)/36 (33.03%)	82 (27.7%)/143 (48.31%)/71 (23.99%)	3.431	0.180
TNM stage (≤ II/> II)	56 (51.38%)/53 (48.62%)	116 (39.19%)/180 (60.81%)	4.843	0.028
Distance from anal verge (≤ 5/> 5)	31 (28.44%)/78 (71.56%)	71 (23.99%)/225 (76.01%)	0.839	0.360
Tumour size (cm)	3.38 ± 0.95	3.52 ± 1.09	1.182	0.238

BMI: Body Mass Index; ECOG: Eastern Cooperative Oncology Group performance status; RIP: the ratio of family income to poverty; TNM: tumor node metastasis classification.

Table 4. Comparison of curative effect between the younger group and the older group

	Younger group (n = 109)	Older group (n = 296)	χ^2	P
			7.756	0.051
CR	7 (6.42%)	6 (2.03%)		
PR	55 (50.46%)	130 (43.92%)		
SD	13 (11.93%)	52 (17.57%)		
PD	34 (31.19%)	108 (36.49%)		

CR: Complete Response; PR: Partial Response; SD: Stable Disease; PD: Progressive Disease.

Table 5. Comparison of irAE occurrences between the younger group and the older group

	Younger group (n = 109)	Older group (n = 296)	χ^2	P
Incidence rate (%)	31 (28.44%)	131 (44.26%)	8.304	0.004
CTCAE (≤ 2/> 2)	76 (69.72%)/33 (30.28%)	153 (51.69%)/143 (48.31%)	10.546	0.001

CTCAE: Common Terminology Criteria for Adverse Events.

Table 6. Comparison of irAE types between the younger group and the older group

	Younger group (n = 109)	Older group (n = 296)	χ^2	P
Arthralgia or myalgia	4 (3.67%)	23 (7.77%)	2.153	0.142
Dermatitis	14 (12.84%)	17 (5.74%)	5.683	0.017
Thyroiditis	6 (5.5%)	51 (17.23%)	9.056	0.003
Colitis	5 (4.59%)	45 (15.2%)	8.296	0.004
Abdominal pain	12 (11.01%)	15 (5.07%)	4.520	0.034
Hepatitis	3 (2.75%)	20 (6.76%)	2.385	0.123
Pancreatitis	2 (1.83%)	11 (3.72%)	0.403	0.526

sured by the CTCAE, also varied significantly between the groups. In the younger group, 69.72% experienced irAEs of grade ≤ 2, whereas in the older group, only 51.69% had irAEs of grade ≤ 2, indicating a greater proportion of the older patients experienced severer irAEs with grades > 2 (χ^2 = 10.546, *P* = 0.001). These findings highlight the increased incidence and severity of irAEs in older rectal cancer patients compared to their younger counterparts.

The incidence of dermatitis was significantly higher in the younger group at 12.84% compared to 5.74% in the older cohort (χ^2 = 5.683, *P* = 0.017) (**Table 6**). Thyroiditis occurred significantly more frequently in older patients, with an incidence of 17.23% versus 5.5% in the younger group (χ^2 = 9.056, *P* = 0.003). Similarly, colitis was more prevalent among older patients at 15.2%, compared to 4.59% in the younger group (χ^2 = 8.296, *P* = 0.004). Moreover, abdominal pain was significantly more common in the younger group at 11.01% versus 5.07% in the older group (χ^2 = 4.520, *P* = 0.034). Other irAEs, including arthralgia or myalgia, hepatitis, and pancreatitis, did not display statistically significant differences between the two age groups (*P* > 0.05). These findings indicate age-related variations in the type of irAEs experienced by rectal cancer patients undergoing immunotherapy.

Risk factors of immune-related adverse events

The ALC was significantly higher in the irAEs group, with a mean of 1.89 × 10^9/L compared to 1.84 × 10^9/L in the no-irAEs group (*t* = 3.111, *P* = 0.002) (**Figure 2B**). Additionally, ALB levels were elevated in the irAEs group, averaging 36.98 g/L versus 35.25 g/L in the no-irAEs group (*t* = 2.595, *P* = 0.010) (**Figure 2D**). The PLR was significantly lower in the irAEs group, with a mean of 129.74 compared to 134.63 in the no-irAEs group (*t* = 2.644, *P* = 0.009) (**Figure 2F**). Other parameters, including White Blood Cell count, Hemoglobin, and MLR, did not show statistically significant differences between the two groups (all *P* > 0.05) (**Figure**

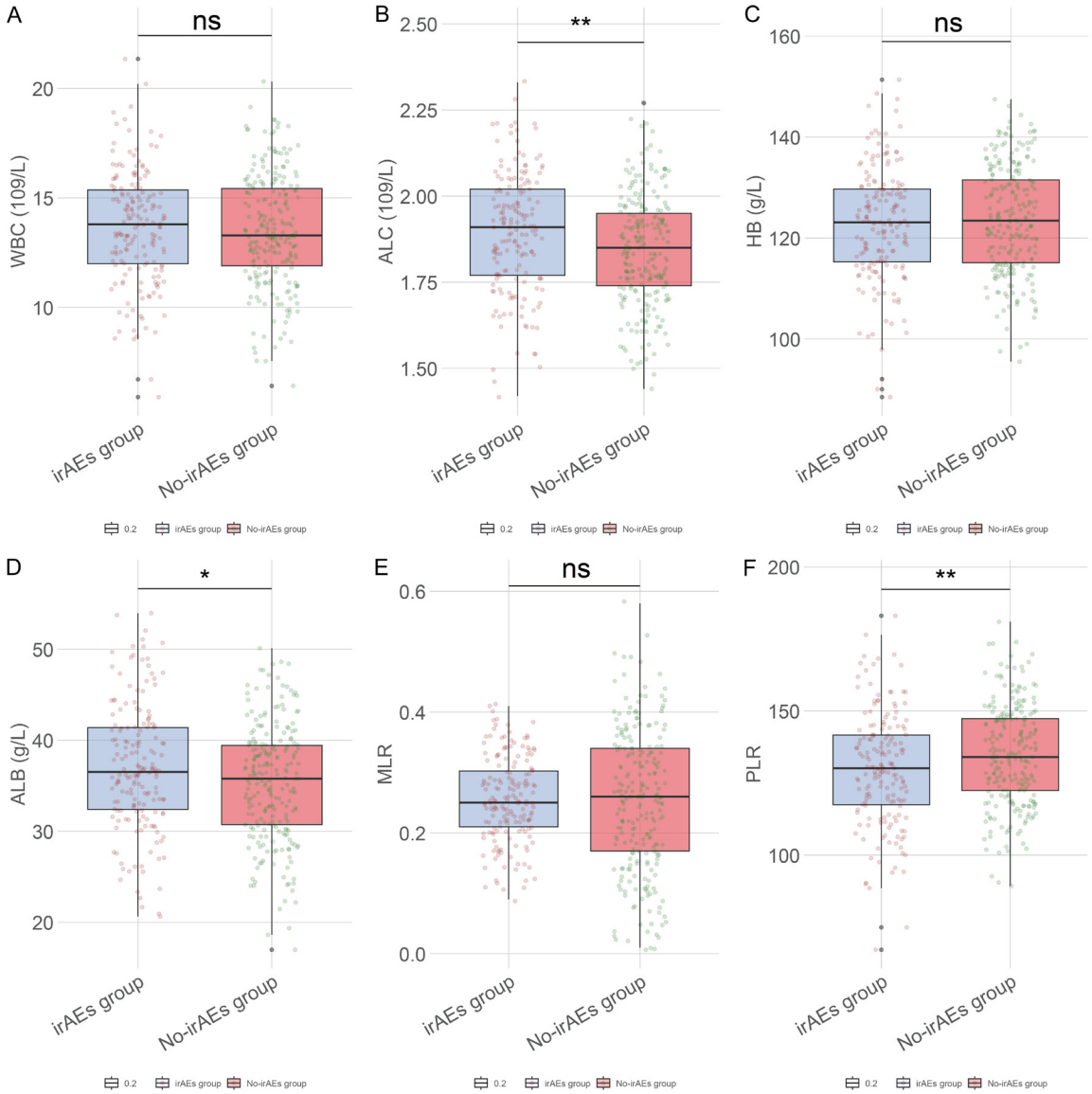

Figure 2. Comparison of blood test results between the No-irAEs group and the irAEs group. *: $P < 0.05$, **$P < 0.01$, ns: no significant difference. A. WBC: white blood cell; B. ALC: absolute lymphocyte count; C. HB: hemoglobin; D. ALB: albumin; E. MLR: monocyte to lymphocyte ratio; F. PLR: platelet-to-lymphocyte ratio.

2A, **2C**, **2E**). These results suggest that ALC, ALB, and PLR may be associated with the occurrence of irAEs in this patient population.

IL-6 levels were significantly higher in the irAEs group, with a mean of 0.35 pg/mL compared to 0.31 pg/mL in the no-irAEs group ($t = 3.232$, $P = 0.001$) (**Figure 3A**). Similarly, IL-22 levels were elevated in the irAEs group, averaging 0.19 pg/mL versus 0.17 pg/mL in the no-irAEs group ($t = 2.295$, $P = 0.022$) (**Figure 3B**). The hypersensitive CRP was also significantly higher in the irAEs group, with a mean of 9.87 mg/L compared to 9.54 mg/L in the no-irAEs group ($t = 2.705$, $P = 0.007$) (**Figure 3C**). PCT levels did not differ significantly between the two groups ($t = 1.791$, $P = 0.074$) (**Figure 3D**). These findings suggest that IL-6, IL-22, and CRP may be associated with the occurrence of irAEs in patients undergoing immunotherapy.

SCF levels were significantly elevated in the irAEs group, averaging 15.44 pg/mL compared to 14.63 pg/mL in the no-irAEs group ($t = 2.540$, $P = 0.012$) (**Figure 4A**). Similarly, levels of BTLA were higher in the irAEs group, with a

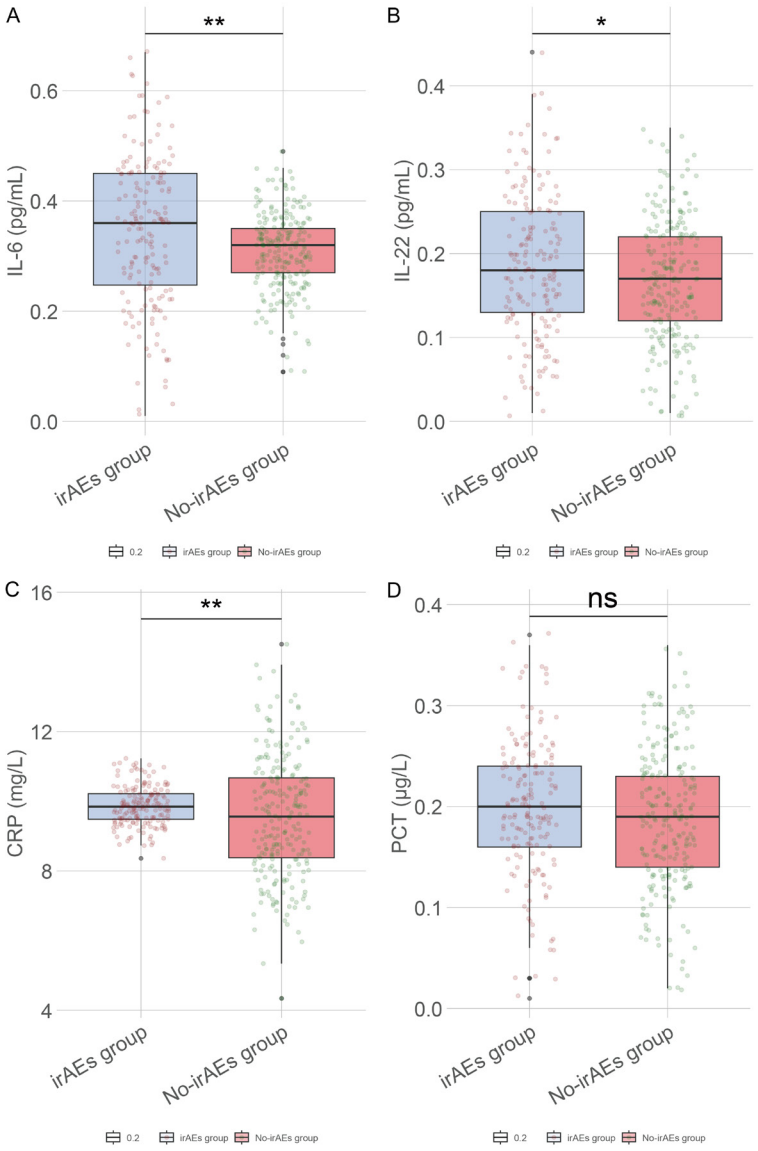

Figure 3. Comparison of inflammatory factor levels in peripheral blood between the No-irAEs group and the irAEs group. *: $P < 0.05$, **$P < 0.01$, ns: no significant difference. A. IL-6: Interleukin-6; B. IL-22: cInterleukin-22; C. CRP: hypersensitive C-reactive protein; D. PCT: Procalcitonin.

PD-1, and PD-L1 were associated with the occurrence of irAEs in this patient population.

Correlation analysis of clinical variables

ALC demonstrated a positive correlation with irAEs (rho = 0.155, $P = 0.002$). ALB levels also showed a positive but weak correlation with irAEs (rho = 0.110, $P = 0.027$) (**Figure 5**). The PLR was inversely correlated with irAEs (rho = -0.121, $P = 0.015$). IL-6 had a notable positive correlation (rho = 0.171, $P < 0.001$), as did BTLA levels (rho = 0.167, $P < 0.001$). Other inflammatory factors such as IL-22, hypersensitive CRP, and SCF also showed significant positive correlations (rho = 0.099, $P = 0.047$; rho = 0.128, $P = 0.010$; rho = 0.136, $P = 0.006$, respectively). Additionally, Human GM-CSF and PD-L1 were positively correlated with the occurrence of irAEs (rho = 0.109, $P = 0.028$; rho = 0.119, $P = 0.016$, respectively). Levels of PD-1 did not show a significant correlation with irAEs (rho = 0.093, $P = 0.060$). These correlations suggest various immune and inflammatory markers were associated with the risk of developing irAEs in this patient population.

mean of 105.22 pg/mL versus 98.37 pg/mL in the no-irAEs group ($t = 3.044$, $P = 0.003$) (**Figure 4B**). The Human GM-CSF also differed significantly, with 0.20 pg/mL in the irAEs group compared to 0.18 pg/mL in the no-irAEs group ($t = 2.319$, $P = 0.021$) (**Figure 4C**). Both PD-1 and PD-L1 levels were significantly higher in the irAEs group, with PD-1 at 44.77 pg/mL versus 42.13 pg/mL ($t = 2.693$, $P = 0.008$) (**Figure 4D**), and PD-L1 at 4.87 pg/mL compared to 4.57 pg/mL in the no-irAEs group ($t = 3.071$, $P = 0.002$) (**Figure 4E**). These findings indicate that elevated levels of SCF, BTLA, GM-CSF,

Univariate analysis of factors affecting the occurrence of irAEs

Univariate logistic regression identified multiple biomarkers linked to irAE risk (**Table 7**). ALC and ALB levels were associated with increased irAE likelihood (OR = 6.683 and 1.040, respectively), whereas higher PLR level demonstrated a protective effect (OR = 0.986). Pro-inflammatory cytokines showed particularly strong associations: IL-6 had the highest risk magnitude (OR = 32.160, P < 0.001), followed by IL-22 (OR = 21.134) and CRP (OR = 1.196).

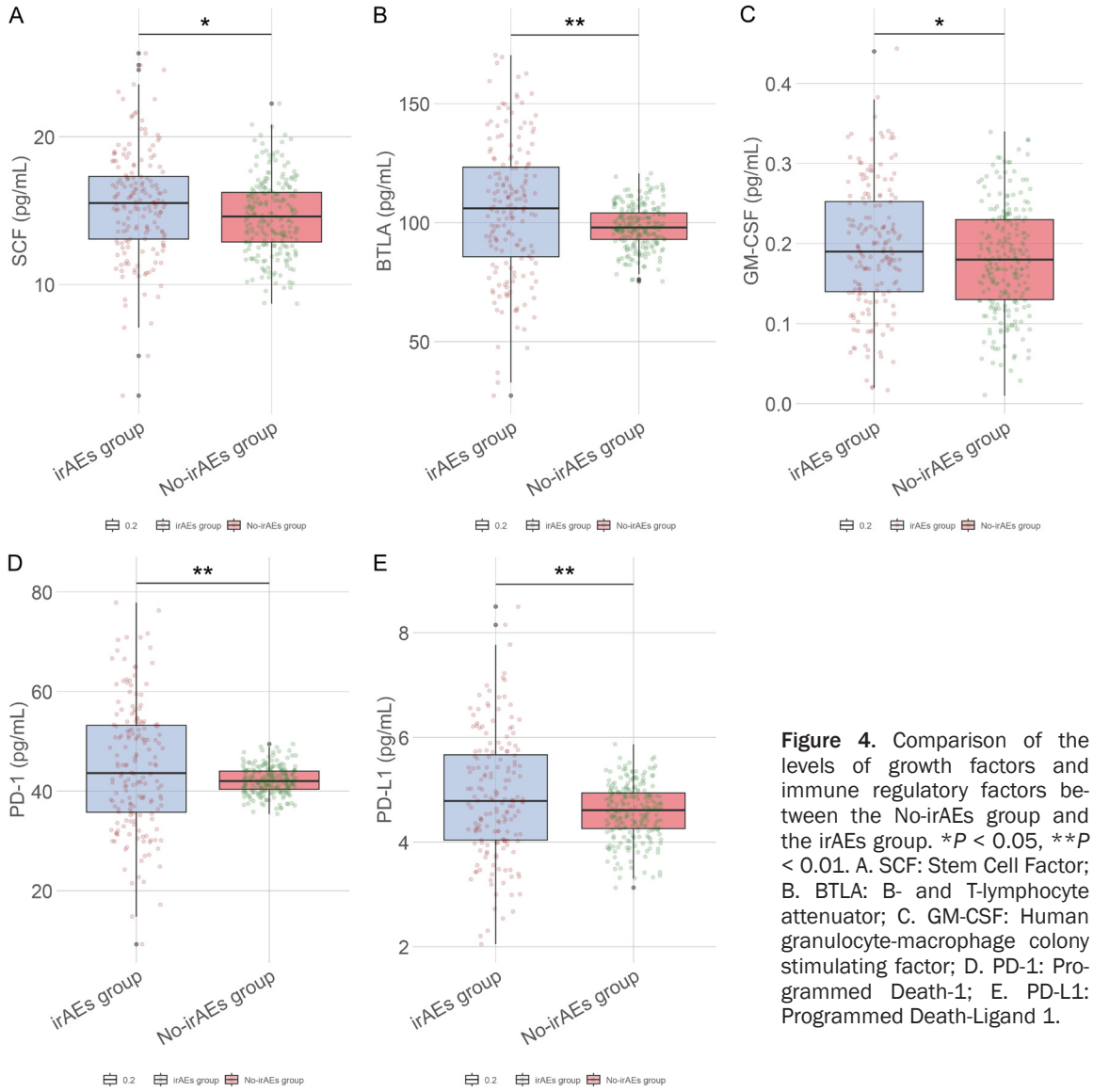

Figure 4. Comparison of the levels of growth factors and immune regulatory factors between the No-irAEs group and the irAEs group. *$P < 0.05$, **$P < 0.01$. A. SCF: Stem Cell Factor; B. BTLA: B- and T-lymphocyte attenuator; C. GM-CSF: Human granulocyte-macrophage colony stimulating factor; D. PD-1: Programmed Death-1; E. PD-L1: Programmed Death-Ligand 1.

Immune checkpoint markers (BTLA, PD-1, PD-L1) and hematopoietic factors (SCF, GM-CSF) were also positively correlated with irAE occurrence ($P < 0.05$ for all).

Multivariate analysis of factors affecting the occurrence of irAEs

Multivariate adjustment confirmed ALC, ALB, IL-6, CRP, BTLA, GM-CSF, PD-1, and PD-L1 as independent predictors for the Occurrence of irAEs (**Table 8**). IL-6 retained the strongest association (OR = 58.961, $P < 0.001$), with a 58-fold increase in irAE risk per unit rise. Notably, PD-L1 exhibited the second-highest effect size (OR = 1.669, $P < 0.001$), while GM-CSF showed wide confidence intervals (OR = 28.799, 95% CI 1.244-666.802), reflecting limited sample stratification. Protective effects of PLR persisted (OR = 0.985, P = 0.013). IL-22 and SCF showed no significance in the adjusted model ($P > 0.10$).

Discussion

The increasing implementation of immunotherapy in managing rectal cancer has brought attention to the occurrence of irAEs, which can exhibit varied incidence and severity across different demographic groups [30-32]. This study aimed to investigate the occurrence of irAEs and associated risk factors with a specific

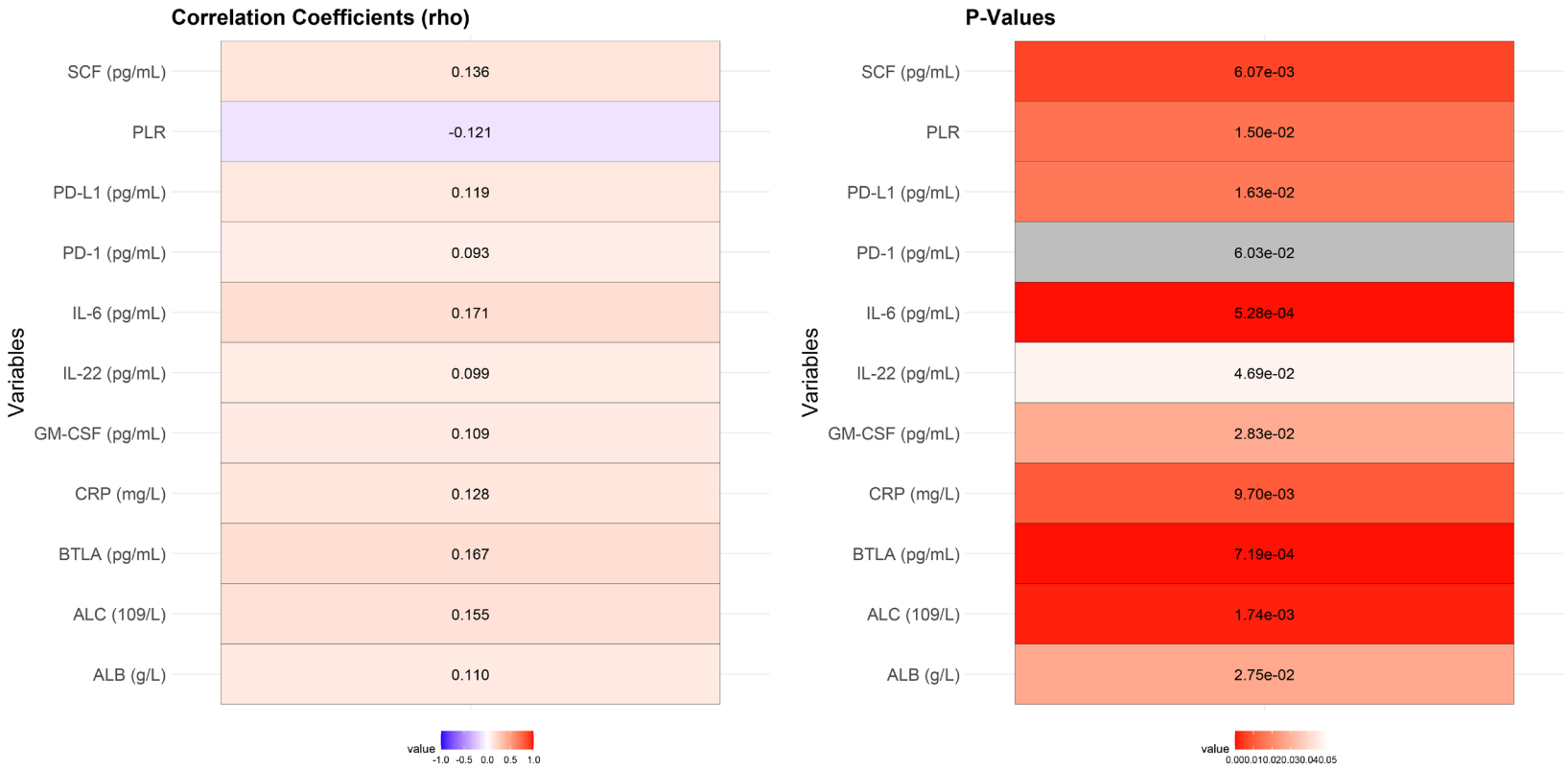

Figure 5. Correlation analysis between influencing factors and irAEs.

Table 7. Univariate analysis of factors affecting the occurrence of irAEs

	Coefficient	Stand Error	Wald	P	OR	95% CI
ALC (10^9/L)	1.900	0.623	3.049	0.002	6.683	1.999-23.110
ALB (g/L)	0.040	0.016	2.560	0.010	1.040	1.010-1.073
PLR	-0.014	0.006	2.605	0.009	0.986	0.975-0.996
IL-6 (pg/mL)	3.471	1.014	3.424	< 0.001	32.160	4.563-245.245
IL-22 (pg/mL)	3.051	1.309	2.331	0.020	21.134	1.657-283.583
CRP (mg/L)	0.179	0.076	2.359	0.018	1.196	1.033-1.391
SCF (pg/mL)	0.089	0.034	2.631	0.009	1.093	1.024-1.170
BTLA (pg/mL)	0.018	0.005	3.331	< 0.001	1.018	1.008-1.029
GM-CSF (pg/mL)	3.352	1.419	2.363	0.018	28.570	1.811-476.860
PD-1 (pg/mL)	0.037	0.012	2.999	0.003	1.038	1.013-1.064
PD-L1 (pg/mL)	0.398	0.121	3.289	0.001	1.488	1.179-1.897

OR: Odds Ratio; CI: Confidence Interval; ALC: Absolute Lymphocyte Count; ALB: Albumin; PLR: Platelet-to-Lymphocyte Ratio; IL-6: Interleukin-6; IL-22: Interleukin-22; CRP: C-Reactive Protein; SCF: Stem Cell Factor; BTLA: B and T Lymphocyte Attenuator; PD-1: Programmed Death-1; PD-L1: Programmed Death-Ligand 1.

Table 8. Multivariate analysis of factors affecting the occurrence of irAEs

	Coefficient	Stand Error	Wald	P	OR	OR CI Lower	OR CI Upper
ALC (10^9/L)	2.272	0.699	3.250	0.001	9.700	2.464	38.178
ALB (g/L)	0.048	0.018	2.698	0.007	1.049	1.013	1.086
PLR	-0.016	0.006	-2.473	0.013	0.985	0.973	0.997
IL-6 (pg/mL)	4.077	1.123	3.629	< 0.001	58.961	6.520	533.159
IL-22 (pg/mL)	2.213	1.492	1.483	0.138	9.146	0.491	170.369
CRP (mg/L)	0.188	0.084	2.237	0.025	1.206	1.023	1.422
SCF (pg/mL)	0.054	0.037	1.449	0.147	1.056	0.981	1.136
BTLA (pg/mL)	0.018	0.006	3.101	0.002	1.019	1.007	1.030
GM-CSF (pg/mL)	3.360	1.603	2.096	0.036	28.799	1.244	666.802
PD-1 (pg/mL)	0.041	0.014	2.997	0.003	1.042	1.014	1.071
PD-L1 (pg/mL)	0.512	0.139	3.681	< 0.001	1.669	1.270	2.192

OR: Odds Ratio; OR CI Lower: Odds Ratio Confidence Interval Lower Bound; OR CI Upper: Odds Ratio Confidence Interval Upper Bound; ALC: Absolute Lymphocyte Count; ALB: Albumin; PLR: Platelet-to-Lymphocyte Ratio; IL-6: Interleukin-6; IL-22: Interleukin-22; CRP: C-Reactive Protein; SCF: Stem Cell Factor; BTLA: B and T Lymphocyte Attenuator; PD-1: Programmed Death-1; PD-L1: Programmed Death Ligand-1.

focus on elderly versus young patients receiving immunotherapy for rectal cancer.

One of the key observations from our research indicates that elderly patients experienced both a higher frequency and increased severity of irAEs compared to their younger counterparts. This can be partially attributed to the age-related decline in immune system function, known as immunosenescence, coupled with inflammaging - a chronic, low-grade inflammation prevalent in older individuals. Immunosenescence may lead to an altered response to immunotherapy, resulting in a heightened susceptibility to adverse immune responses [33-35]. This phenomenon is compounded by inflammaging, a hallmark of aging marked by chronic IL-6 and CRP elevation due to senescence-associated secretory phenotype cells and mitochondrial dysfunction [36]. In contrast, younger patients typically possess a more robust immune response, which might account for the observed lower incidence rate and milder irAEs. Nevertheless, specific irAEs such as dermatitis presented more prominently in younger individuals, hinting at possible differences in the types of immune responses activated across age groups.

The observed variation in specific irAEs types, like higher rates of dermatitis in younger patients and thyroiditis in older ones, might be

related to age-associated differential expression of immune checkpoint molecules or variations in immune cell populations. Younger patients' immune systems may react more aggressively to immunotherapies, potentially triggering dermatological irAEs due to an enhanced T cell activation profile. Conversely, older patients may experience thyroiditis more frequently, possibly due to their existing vulnerability to autoimmune conditions driven by shifts in autoimmune regulatory mechanisms with age. This differential susceptibility highlights the necessity of age-specific monitoring and management strategies in immunotherapy regimens.

Our analysis also identified critical biomarkers associated with irAEs occurrence, with elevated levels of ALC, IL-6, CRP, BTLA, GM-CSF, PD-1, and PD-L1 emerging as significant predictors. The strong association between higher ALC and irAEs suggests that an increased proliferation of lymphocytes might play a role in the pathogenesis of these events [37]. High ALC may reflect clonal expansion of autoreactive T-cells post-ICI treatment, as demonstrated in murine models where PD-1 blockade expanded CD8+ T-cell clones cross-reactive with self-antigens [38]. Elevated lymphocyte counts may indicate a hyperactive immune state aggravated by immunotherapies, fostering an environment conducive to adverse reactions [5]. Moreover, IL-6, a cytokine critically involved in inflammation and immune modulation, was markedly elevated in patients experiencing irAEs [39]. CRP, an acute-phase reactant induced by IL-6, further amplifies tissue damage through complement activation and endothelial dysfunction [40], creating a feedforward loop of inflammation. This finding supports the notion that an inflammatory milieu, characterized by increased cytokine levels, might exacerbate or precipitate immune-related toxicities. Furthermore, elevated levels of pro-inflammatory markers like CRP and IL-22 reaffirm the role of inflammation in irAEs. These markers not only signal ongoing inflammation but also suggest a robust immune activation potentially leading to tissue damage and adverse events. Consequently, strategies to modulate these inflammatory processes could mitigate irAE development, providing a therapeutic window for intervention, especially in high-risk populations.

Interestingly, the study also highlighted the protective role of the PLR, suggesting its utility as a potential biomarker for monitoring and mitigating irAE risk. A lower PLR in patients with irAEs might reflect an imbalance in hematopoietic homeostasis or an underlying pro-inflammatory state, emphasizing the need for integrative approaches in managing immune parameters alongside cancer therapies. The protective effect of low PLR aligns with recent findings that thrombocytosis promotes immunosuppression via TGF-β release and Treg induction [41]. In irAE-prone patients, reduced PLR may indicate platelet consumption due to microvascular inflammation or enhanced megakaryocyte-erythroid progenitor differentiation bias under cytokine stress, both warranting further lineage-tracing studies.

Higher expressions of immune inhibitory receptors, such as PD-1 and PD-L1, further underline the interplay between immune activation and suppression pathways modulating irAE risk. These molecules, crucial in immune checkpoint pathways targeted by immunotherapy, may reflect a state of ongoing immune surveillance and regulation [42]. Their elevated levels in patients with irAEs support the hypothesis of a dysregulated immune landscape, where ICIs disrupt the delicate balance between activation and inhibition, precipitating adverse immune reactions. The presence of elevated growth factors and immune modulators like GM-CSF and BTLA in irAE cases further elucidates the complex network of immune regulation in the context of immunotherapy. GM-CSF, a key player in immune cell maturation and activation, may contribute to excessive immune activation when dysregulated or overexpressed, leading to systemic irAEs [43]. Similarly, BTLA, a co-inhibitory receptor, might reflect compensatory mechanisms aiming to curtail excessive immune responses in the face of rising irAE incidences [44].

Overall, our findings suggest a multifaceted approach to understanding irAEs, involving not only patient characteristics such as age but also intricate immunological landscapes. Tailoring immunotherapy regimens based on these parameters could enhance therapeutic efficacy while minimizing adverse effects. The elucidation of specific markers and immunological profiles associated with irAEs allows for the poten-

tial development of predictive tools and personalized interventions. These could include pre-treatment evaluation of immune markers or cytokine profiles to identify high-risk patients, implementing prophylactic measures, or employing adjunctive therapies to modulate the immune system's response.

While our study provides significant insights, further research was warranted to dissect the exact molecular and cellular mechanisms underpinning these observations. Longitudinal studies assessing immune and inflammatory biomarkers in diverse patient populationsenrich our understanding of irAE pathogenesis and guide adaptive therapeutic strategies. Additionally, exploring genetic predispositions and incorporating multi-omics approaches could unravel the complexities of immune responses in cancer patients undergoing immunotherapy.

While this study offers valuable insights into the differential occurrence of irAEs between elderly and young rectal cancer patients receiving immunotherapy, several limitations must be acknowledged. First, the study's retrospective design could introduce selection bias and limit the ability to establish causal relationships. The sample size, while adequate for initial observations, may not fully capture the diversity within age groups, particularly concerning genetic and environmental factors that could influence immune responses. Additionally, the reliance on hospital records for data collection might have led to incomplete documentation of irAEs, potentially underestimating their true incidence and severity. The study also did not extensively explore the impact of concurrent medications or comorbidities, which could confound the results. Finally, while biomarkers were identified as potential predictors of irAEs, the cross-sectional nature of the analysis precludes conclusions about their prognostic value over time. Future research involving prospective studies with larger and more diverse populations, alongside mechanistic exploration through multi-omics approaches, would be beneficial to validate and expand upon these findings.

Conclusion

In conclusion, our research underlines the critical impact of age on irAE manifestation in rectal cancer patients undergoing immunotherapy and identifies key hematologic and immunologic predictors of these adverse events. The insights gained have paved the way for more personalized and safer immunotherapy approaches, emphasizing the importance of integrating demographic and biological data in clinical decision-making.

Disclosure of conflict of interest

None.

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Supplementary Table 1. The Eastern cooperative oncology group definitions

Classification	Definition
0	Fully active, with no restrictions in activity compared to before the onset of the disease.
1	Ambulatory and able to conduct light physical activities, including light housework or office work, but unable to perform heavy physical labor.
2	Mobile and self-sufficient, yet unable to hold a job; active for the majority of waking hours.
3	Able to perform only minimal self-care, spending more than half of the waking hours in bed or seated.
4	Completely disabled, confined to bed or a chair, and unable to care for oneself.
5	Deceased.

Supplementary Table 2. TNM staging system definitions

Classification	Definition
T (Tumor)	It evaluates the size and extent of the primary tumor, categorized into four levels: T1, T2, T3, and T4. A higher T value signifies a larger tumor with more extensive invasion.
N (Node)	It reflects the involvement of regional lymph nodes and was divided into four categories: N0, N1, N2, as well as N3, with higher numbers indicating a greater degree of lymph node involvement.
M (Metastasis)	It indicates whether distant metastasis was present, with M0 denoting the absence of metastasis and M1 denoting its presence.

TNM: tumor node metastasis classification.

Supplementary Table 3. Blood collection and biomarker analysis methods

Category	Parameter/Procedure	Method/Instrument	Specifications	Manufacturer
Sample Collection	Venous & arterial blood collection	Fasting patients	-Volume: 5 mL (venous + arterial) -Time: Within 24 h of admission	-
Sample Processing	Plasma separation	Low-temperature high-speed centrifuge	-Speed: 3000 rpm -Duration: 10 min -Storage: -80 °C	TLD 12A, Hunan Xiangxi Scientific Instrument Factory, China
Hematology	WBC, ALC, HB, ALB	Automated hematology analyzer		Mindray BC6800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China
Calculated Ratios	Monocyte-to-lymphocyte ratio (MLR) Platelet-to-lymphocyte ratio (PLR)	Derived from hematology parameters	-Formula: MLR = Monocyte count/Lymphocyte count PLR = Platelet count/Lymphocyte count	-
Biochemical Assays	IL-6, IL-22, CRP, PCT, SCF, BTLA, PD-1, PD-L1	Automated biochemistry analyzer	-	AU5811, Shanghai Kehua Bio-Engineering Co., Ltd., China
Immunoassay	GM-CSF	Enzyme-linked immunosorbent assay (ELISA)	-	YS01118B, Shanghai Yaji Biotechnology Co., Ltd., China

WBC: White Blood Cell count; ALC: Absolute Lymphocyte Count; HB: Hemoglobin; ALB: Albumin; PLR: Platelet-to-Lymphocyte Ratio; IL-6: Interleukin-6; IL-22: Interleukin-22; CRP: C-Reactive Protein; SCF: Stem Cell Factor; BTLA: B and T Lymphocyte Attenuator; PD-1: Programmed Death-1; PD-L1: Programmed Death-Ligand 1; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor.