Therapeutics and Clinical Risk Management

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ORIGINAL RESEARCH

Clinical Manifestations and Risk Factors of Liver Injury Induced by PD-1 Inhibitors in Patients with Malignancies: A Case-Control Study

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Background: Hepatic injury induced by immune checkpoint inhibitors (ICPIs) is an inevitable challenge in the era of innovative anti-tumor therapies. However, studies on immune-related liver injury are relatively insufficient, and the associated risk factors are still lacking. The purpose of this study was to explore the incidence and clinical manifestations of immunotherapy-related liver injury.

Methods: A retrospective case-control study was conducted involving patients treated with PD-1 inhibitors at Weifang People's Hospital, a tertiary general hospital in China, from January 1, 2021 and July 31, 2024. Univariate and multivariate logistic regression analyses were employed to identify the potential risk factors. Then, the predictive value of these risk factors was evaluated using receiver operating characteristic (ROC) curve analysis.

Results: In total, 300 patients were included. Among these patients, 52 patients experienced liver injury. The mean time from the initiation of immunotherapy to the onset of liver injury was 28.4 days, with a range from 2 to 219 days. 71.15% of patients developed liver injury within the first 30 days. 82.69% presented with mild cases (grade 1), 13.46% with moderate cases (grade 2), and 3.84% with severe cases (grades 3–4). The overall incidence of PD-1 inhibitors-related liver injury was 0.34%. Specifically, nivolumab exhibited the highest incidence at 2.86%, followed by sintilimab at 0.41%. Both toripalimab and camrelizumab exhibited an incidence of 0.34%, while tislelizumab had the lowest at 0.28%. Multivariate logistic regression analysis showed that GGT and AST were independent risk factors for liver injury. ROC curve analysis revealed that patients with baseline ALT≥19.5 U/L, AST≥19.5 U/L, and GGT≥28.5 U/L were at increased risk of developing liver injury.

Conclusion: In clinical therapy, close monitoring of liver function is recommended, especially for patients with baseline ALT≥19.5 U/L, AST≥19.5 U/L, and GGT≥28.5 U/L during immunotherapy with PD-1 inhibitors.

Keywords: PD-1 inhibitors, immune-related adverse events, liver injury, incidence, risk factors

Introduction

Immune checkpoint inhibitors (ICPIs) have emerged as an important cancer treatment method following surgery, radiotherapy, chemotherapy, and molecular targeted therapy. By blocking negative regulatory factors that suppress T cell function, ICPIs activate T cells, harnessing the body's own immune system to combat cancer.[1] In recent years, immunotherapy, represented by programmed death-1 (PD-1) inhibitors, has become a significant milestone in cancer treatment. Due to the specific mechanism of ICPIs, there is a lack of selectivity between tumor cells and normal cells. The activated immune system, while attacking tumor cells, also downregulates tolerance to self-antigens, leading to potential immune-related adverse events (irAEs) in normal tissues.[2] Nearly two-thirds of patients undergoing treatment with ICPIs experience irAEs to varying degrees, and these events can occur at any time.[3] Most irAEs are relatively mild and can be reversible with early identification and intervention, however, without prompt management of acute events, they may become life-threatening.[4]

Received: 7 December 2024
Accepted: 14 February 2025
Published: 11 March 2025

The liver, as a unique immune and detoxification organ, is one of the most commonly affected organs in tumor immunotherapy. Immune-mediated liver injury caused by checkpoint inhibitors (ILICI) differs from classical drug-induced liver injury (DILI) in terms of incidence, clinical presentation, pathogenesis, and prognosis.[5] The hepatotoxicity induced by ICPIs in tumor immunotherapy is immune-mediated, but the exact pathophysiological mechanisms remain unclear. It may be related to T cell activation induced by ICPIs, leading to increased autoimmunity against hepatocytes. The reported incidence of ILICI varies across different studies. Remash et al[6] reported the overall incidence of ILICI fluctuates between 0% and 30%, with grades 3/4 occurring at rates of 0% to 20%. The incidence of liver failure or death is approximately 0.4%, with ILICI-related deaths accounting for 16% of all ICPIs-related fatalities. The incidence of ILICI increases with combination therapies, with overall liver injury rates and grades 3/4 liver injury rates reported at 18% to 22% and 8% to 11%, respectively. ILICI typically occurs 4 to 12 weeks after the initial administration or after 1 to 3 doses of the drug.[7,8] The most common manifestation of ILICI is asymptomatic elevation of liver enzymes, and some patients may experience symptomatic elevations accompanied by fever, fatigue, nausea, abdominal discomfort, and rash. In severe cases, symptoms may progress to jaundice and coagulopathy, potentially leading to life-threatening acute liver failure.[9]

Current research on PD-1 inhibitors primarily focuses on their efficacy. With the broader application of PD-1 inhibitors, there is growing concern about their associated adverse reactions. Hepatotoxicity during PD-1 inhibitor therapy presents a new challenge in the field of drug-induced liver injury. In recent years, reports of immune-mediated liver injury caused by PD-1 inhibitors have gradually increased, however, due to the relatively short time since the introduction of PD-1 inhibitors in China, the available data on drug use in Chinese patients is insufficient. Most data on PD-1 inhibitor-related adverse reactions stem from clinical trials, with real-world reports of PD-1 inhibitor-related ILICI largely consisting of individual case reports. Currently, the few available studies on the factors influencing liver injury associated with PD-1 inhibitors yield inconsistent results. Based on active surveillance from the Chinese Hospital Pharmacovigilance System (CHPS), we retrospectively investigated PD-1 inhibitors-associated liver injury in real-world patients undergoing anti-PD-1 therapy. Potential clinical factors that may increase susceptibility to this adverse event were identified and quantified. The study process was outlined in Figure 1.

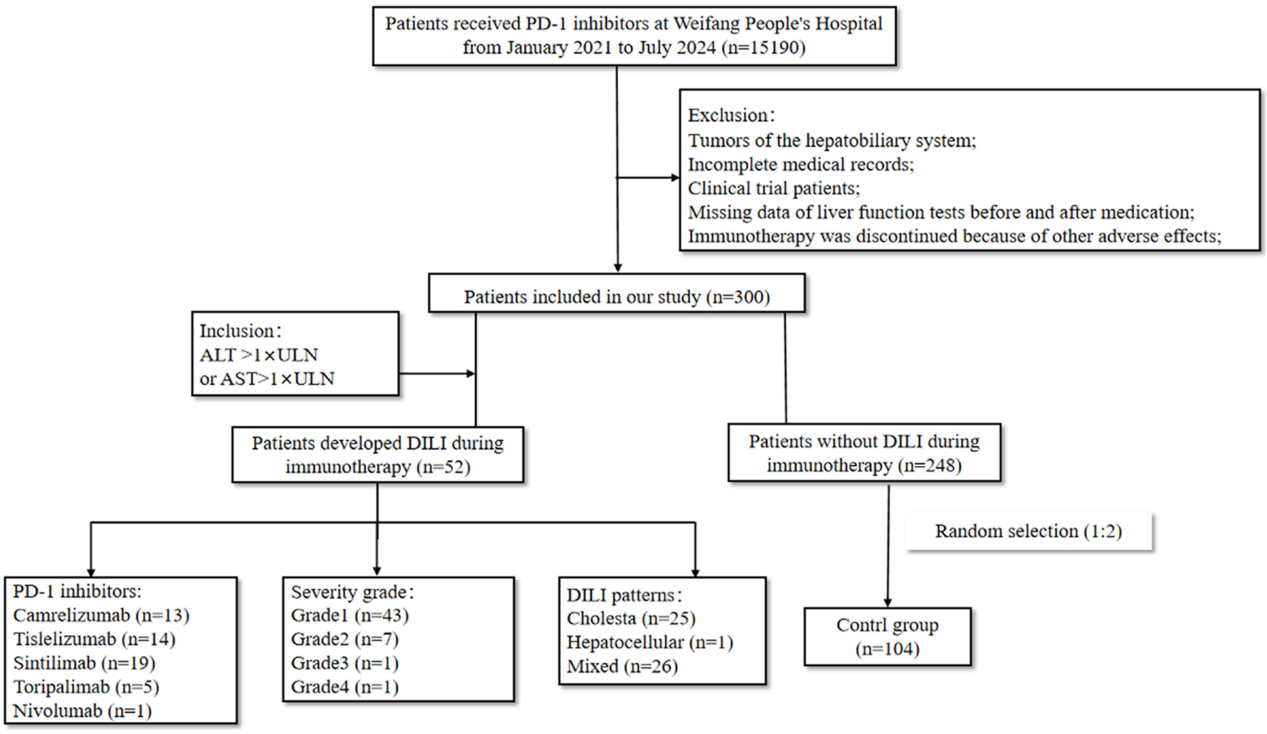

Figure 1 The technical flow chart of this study.

Methods

Study Population and Data Collection

This retrospective, single-center, observational study was conducted at Weifang People's Hospital, a tertiary general hospital in China. Patients data was extracted from the Hospital Information System (HIS) between January 1, 2021 and July 31, 2024. PD-1 inhibitors included camrelizumab, tislelizumab, sintilimab, toripalimab, nivolumab and pembrolizumab. Patients treated with PD-1 inhibitors but not have liver injury during hospitalization were selected as the control group. The medical records of enrolled patients were meticulously reviewed, and the following data were extracted for each patient: age, gender, smoking and drinking status, comorbidities, tumor type and stage, the type, dose and frequency of PD-1 inhibitors treatment, the occurrence time, symptoms, and grading of liver injury, baseline clinical laboratory test results prior to the first day of PD-1 inhibitors treatment, including liver function indicators [alanine transaminase (ALT), aspartate transaminase (AST), direct bilirubin (DBil), total bilirubin (TBil), albumin (ALB), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total protein (TP)], renal function indicators [serum creatinine (SCR), blood urea nitrogen (BUN)], coagulation function indicators [thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio of prothrombin time (INR), fibrinogen (Fib), D-Dimer (D-D)], platelet [PLT], white blood cell (WBC), hemoglobin (HGB), the percent of neutrophile granulocyte (GRAN). The occurrence of drug-induced liver injury involves various medications, and therefore we selected statins, proton pump inhibitors, and heparin (including heparin sodium, dalteparin sodium, nadroparin and low molecular heparin) as concomitant drugs. During the use of PD-1 inhibitors, concomitant use may be considered if the prescription period overlaps for more than one day. For liver injury group, we also evaluated the time from the initiation of PD-1 inhibitors therapy to the occurrence of liver injury and severity. Additionally, the treatment (hepatoprotective drugs or corticosteroid therapy) and outcomes of patients with liver injury were assessed.

Adverse Events Definitions and Severity Grading

The CHPS system was capable of identifying patients at risk of developing liver injury by utilizing predefined inclusion and exclusion criteria to issue early warning alerts, which were subsequently subjected to manual evaluation for confirmation. According to the 2021 Chinese Society of Clinical Oncology (CSCO) Guidelines for the Management of Immune Checkpoint Inhibitor-Related Toxicity, the diagnostic criteria for immune checkpoint inhibitors-related liver injury was defined as ALT or AST>1×ULN. The severity of liver injury was stratified into four grades: grade 1 (ALT or AST>1×ULN), grade 2 (ALT or AST 3–5×ULN), grade 3 (ALT or AST 5–20×ULN), and grade 4 (ALT or AST>20×ULN). Among them, grade 1 is classified as mild, grade 2 is classified as moderate, and grade 3 and 4 are classified as severe. Patients with incomplete laboratory test results or medical records, primary liver cancer, liver function results outside of the normal range prior to immunotherapy and absence of baseline information or follow-up liver function data were excluded from the study.

Cases Assessment

Two clinical pharmacists independently performed a blinded assessment using the Roussel Uclaf Causality Assessment Method (RUCAM) to evaluate the causality in alarmed patients. The scores are divided into highly probable (>8), probable (6–8), possible (3–5), unlikely (1–2). Patients with RUCAM score ≥6 were directly included in the case group. For patients scoring 3–5, the researchers re-evaluated the cases back-to-back based on the patient's medical history. Patients with consistent results were classified as positive cases, while cases with discrepancies were referred to experts for final judgment. Cases with RUCAM scores of less than 3 were directly excluded. The pattern of liver injury was determined based on the R values calculated from liver function tests (R=[ALT/ALT ULN]/[ALP/ALP ULN]), classifying the injury as hepatocellular (R≥5), cholestatic (R≤2) and mixed (2<R<5). The R values were calculated at the time when liver enzyme levels first reached the warning threshold following immunotherapy.

Statistical Analysis

All the statistical analyses were performed using SPSS statistics software 26.0. Continuous variables with a normal distribution were expressed as mean ± standard deviation (SD), while those with a non-normal distribution were presented as median and interquartile range (Q1, Q3). Categorical variables were reported in terms of counts and percentages. The time to reach liver injury was analyzed using the Kaplan-Meier curves. The Chi-square test or Fisher exact test was used to compare categorical variables between patients with and without liver injury. The Mann–Whitney *U*-test was used to analyze two independent groups, while the Kruskal–Wallis *H*-test was applied for multiple independent groups. Variables with significance in univariate analysis were subsequently analyzed using binary logistic regression analysis to identify independent risk factors associated with liver injury. The adjusted odd ratios (OR) and 95% confidence intervals (CI) were calculated for each variable. The predictive value and threshold of relevant risk factors for the occurrence of liver injury were analyzed using the receiver operating characteristic (ROC) curve. Statistical significance was established at $p<0.05$.

Ethics Approval

This study was approved by the Ethics Committee of Weifang People's Hospital (KYLL20230823-4). All procedures were carried out in strict accordance with relevant guidelines and regulations, and adhered to the principles of the Declaration of Helsinki. Due to the retrospective nature of the study and the anonymization of patient data, the Ethics Committee granted a waiver for informed consent. At no point during or after data collection were the authors able to access any information that could identify individual participants.

Results

Basic Patient Information

With the help of CHPS active monitoring system, we extracted 15190 patients who received PD-1 inhibitors therapy between January 1, 2021, and July 31, 2024. Of these, 300 cases (male-to-female ratio, 4.1:1) were alarmed by the system. Based on the inclusion and exclusion criteria, 52 patients were ultimately enrolled in liver injury group. The remaining 248 patients were classified into the non-liver injury group, from which a subset of 104 patients were randomly selected as the control group. Of the 156 patients (52 patients in liver injury group and 104 patients in non-liver injury group) analyzed in this study, 125 patients were male and 31 were female, with a male-to-female ratio of 4.03:1. In the present study, the incidence of liver injury associated with PD-1 inhibitors was 0.34%. Of the 52 patients in the liver injury group, 42 patients (80.8%) were male and 10 (19.2%) were female, yielding a male-to-female ratio of 4.2:1. The median age of the patients was 62.5 (57.0, 68.0) years, with an age range of 27 to 88 years. Patients over 50 years old accounted for 86.5% (Figure 2a). A total of five PD-1 inhibitors were involved, including nivolumab (1 case), toripalimab (5 cases), camrelizumab (13 cases), tislelizumab (14 cases) and sintilimab (19 cases). Of the 52 patients diagnosed, 16 patients suffered from lung cancer, accounting for 30.8%, followed by 15 patients (28.8%) with gastric cancer, 7 patients (13.5%) with esophageal cancer, 3 patients (5.8%) with bladder cancer, and the remaining 11 patients (21.2%) were afflicted with various other tumor types.

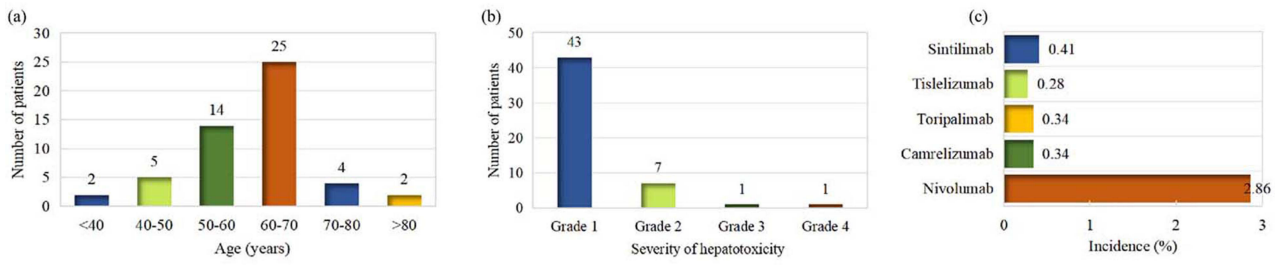

Figure 2 The age of the patients (**a**), as well as the severity (**b**) and the incidence (**c**) of liver injury.

Severity, Onset Time and Incidence of Liver Injury

In general, the majority of patients exhibited mild to moderate liver injury (Figure 2b), with 43 cases (82.69%) being mild (grade 1) and 7 cases (13.46%) being moderate (grade 2). Severe liver injury (grade 3 and 4) was relatively uncommon, with only 2 cases (3.84%). In terms of incidence, nivolumab exhibited the highest occurrence of liver injury at 2.86%, followed by sintilimab at 0.41%. Both toripalimab and camrelizumab exhibited an incidence of 0.34%, while tislelizumab had the lowest at 0.28% (Figure 2c). The Kaplan-Meier curve of the time to the onset of liver injury following the initiation of PD-1 inhibitors therapy were presented in Figure 3. Overall, the mean time from the commencement of immunotherapy to the manifestation of liver injury was 28.4 days, with a range from 2 to 219 days. Notably, 37 cases (71.15%) developed liver injury within the first 30 days (Figure 3a). The median time to the onset of grade 1 liver injury was 16 (3, 33) days, whereas the median time for the onset of grade 2 or higher liver injury was 8 (4.5, 47) days (Figure 3b).

Treatment and Prognosis of Liver Injury

Among the 52 patients, 39 were administered liver-protecting medications (such as magnesium isoglycyrrhizinate injection, bicyclol tablets, glutathione, and polyene phosphatidylcholine capsules), glucocorticoids (such as methylprednisolone and prednisone), or a combination of both following the onset of liver injury. The clinical characteristics of liver injury patients were categorized based on severity (Table 1). It can be observed that, among the 43 patients with grade 1 liver injury, 32 (74.4%) received hepatoprotective treatment, among the 9 patients with grade 2 or higher liver injury, 1 (11.1%) received steroid therapy, 4 (44.4%) received hepatoprotective treatment, and 2 (22.2%) received both hepatoprotective and steroid treatments. In terms of prognosis, the liver function of 39 patients was fully recovered, while 3 patients experienced partial improvement, and an aggravation was found in 7 patients. Specifically, among the patients with grade 1 liver injury, 36 (83.7%) achieved full recovery of liver function, 1 (2.3%) showed improvement, and 3 (7.0%) experienced a deterioration in their liver condition. Of the 9 patients with grade 2 or higher liver injury, 3 (33.3%) fully recovered after medical intervention, 2 (22.2%) showed improvement, and 4 (44.4%) experienced a worsening of liver injury.

Comparison of the Clinical Characteristics Between Liver Injury and Non-Liver Injury Group

As shown in Table 2, we compared the patients' baseline characteristics, laboratory parameters, hepatic and renal function, comorbidities, and concomitant medications between patients with and without liver injury groups. Univariate analysis showed that several factors were found to be significantly associated with the occurrence of liver injury, including age (p=0.013), GGT (p<0.001), ALT (p<0.001), and AST (p<0.001). Conversely, no statistically significant differences were observed between the liver injury group and the control group for other indicators, including gender,

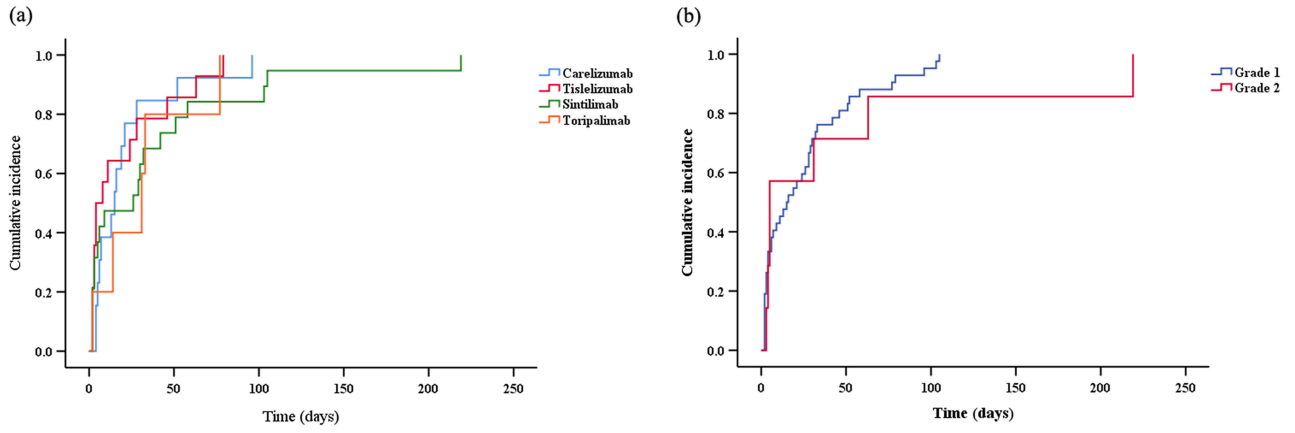

Figure 3 The cumulative incidence curve of the time to the onset of liver injury after the initiation of PD-1 inhibitors therapy depending on: (**a**) the type of PD-1 inhibitors; (**b**) the severity of liver injury.

https://doi.org/10.2147/TCRM.S510973

Table 1 Clinical Characteristics of Patients with Liver Injury at Different Degrees

Characteristics	Grade 1 (n=43)	Grade ≥2 (n=9)
Age (year), mean ± SD	61.53±9.11	61.44±17.45
Gender, n (%)		
Male	35 (81.4%)	7 (77.8%)
Female	8 (18.6%)	2 (22.2%)
Hypertension, n (%)		
Yes	11 (25.6%)	3 (33.3%)
No	32 (74.4%)	6 (66.7%)
Diabetes mellitus, n (%)		
Yes	4 (9.3%)	1 (11.1%)
No	39 (90.7%)	8 (88.9%)
Smoking, n (%)		
Yes	27 (62.8%)	5 (55.6%)
No	16 (37.2%)	4 (44.4%)
Alcohol drinking, n (%)		
Yes	20 (46.5%)	1 (11.1%)
No	23 (53.5%)	8 (88.9%)
Combination chemotherapy, n (%)		
Yes	42 (97.7%)	7 (77.8%)
No	1 (2.3%)	2 (22.2%)
Hepatic metastasis, n (%)		
Yes	8 (18.6%)	1 (11.1%)
No	35 (81.4%)	8 (88.9%)
Concomitant medications, n (%)		
Statins	3 (7.0%)	2 (22.2%)
PPIs	16 (37.2%)	5 (55.6%)
Heparins	5 (11.6%)	2 (22.2%)
Laboratory data at the baseline		
PT (S), M (Q25, Q75)	12.90 (12.30, 13.60)	12.80 (12.20, 13.30)
INR, M (Q25, Q75)	1.06 (1.01, 1.12)	1.05 (1.01, 1.12)
Fib (g/L), mean ± SD	3.88±1.10	3.97±1.13
TT (S), M (Q25, Q75)	15.70 (15.30, 16.50)	15.90 (15.25, 16.30)
APTT (S), mean ± SD	27.97±3.42	26.96±2.60
D-D (μg/mL), M (Q25, Q75)	1.08 (0.69, 2.55)	1.13 (0.65, 9.60)
WBC (10^9/L), M (Q25, Q75)	6.33 (4.92, 7.97)	5.05 (4.16, 8.04)
HGB (g/L), mean ± SD	117.86±20.64	121.56±22.25
PLT (10^9/L), M (Q25, Q75)	239.00 (181.00, 306.00)	262.00 (186.00, 367.00)
GRAN (%), mean ± SD	67.04±11.80	63.20±12.15
TP (g/L), mean ± SD	64.65±6.37	66.69±6.42
GGT (U/L), M (Q25, Q75)	36.00 (21.00, 57.00)	32.00 (17.00, 42.00)
ALT (U/L), mean ± SD	22.12±10.72	22.22±13.75
AST (U/L), mean ± SD	23.00±7.24	20.67±6.69
ALB (g/L), mean ± SD	39.16±5.25	38.94±5.67
DBil (umoL/L), M (Q25, Q75)	3.70 (2.70, 5.00)	3.30 (1.95, 4.55)
TBil (umoL/L), M (Q25, Q75)	11.60 (7.70, 14.00)	9.00 (7.10, 14.40)
ALP (U/L), M (Q25, Q75)	80.00 (60.00, 108.00)	76.00 (65.50, 92.00)
SCR (μmol/L), M (Q25, Q75)	57.00 (51.00, 66.00)	67.00 (41.50, 84.50)
BUN (mmol/L), mean ± SD	5.39±2.08	4.83±1.77

(*Continued*)

Table 1 (Continued).

Characteristics	Grade 1 (n=43)	Grade ≥2 (n=9)
PD-1 inhibitors, n (%)		
Carelizumab	11 (25.6%)	2 (22.2%)
Tislelizumab	12 (27.9%)	2 (22.2%)
Sintilimab	16 (37.2%)	3 (33.3%)
Toripalimab	3 (7.0%)	2 (22.2%)
Nivolumab	1 (2.3%)	0 (0.0%)
Pre-existing liver disease, n (%)		
Hepatic cyst	5 (11.6%)	1 (11.1%)
Hepatitis B	1 (2.3%)	0 (0.0%)
Primary tumour type, n (%)		
Lung cancer	12 (27.9%)	4 (44.4%)
Gastric cancer	12 (27.9%)	3 (33.3%)
Esophagus cancer	7 (16.3%)	0 (0.0%)
Bladder cancer	3 (7.0%)	0 (0.0%)
Tumor stage 4, n (%)	21 (48.8%)	3 (33.3%)
Treatment of hepatotoxicity, n (%)		
Corticosteroid	0 (0.0%)	1 (11.1%)
Hepatoprotectant	32 (74.4%)	4 (44.4%)
Hepatoprotectant+Corticosteroid	0 (0.0%)	2 (22.2%)
Without treatment	11 (25.6%)	2 (22.2%)
Outcome, n (%)		
Resolution	36 (83.7%)	3 (33.3%)
Improvement	1 (2.3%)	2 (22.2%)
Aggravation	3 (7.0%)	4 (44.4%)
Duration from immunotherapy to liver injury onset days (IQR)	16.00 (3.00, 33.00)	8.00 (4.50, 47.00)

Abbreviations: PPIs, proton pump inhibitors; ALT, alanine transaminase; AST, aspartate transaminase; DBil, direct bilirubin; TBil, total bilirubin; ALB, albumin; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TP, total protein; SCR, serum creatinine; BUN, blood urea nitrogen; TT, thrombin time; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio of prothrombin time; Fib, fibrinogen; D-D, D-Dimer; PLT, platelet; WBC, white blood cell; HGB, hemoglobin; GRAN, the percent of neutrophile granulocyte.

Table 2 Comparison of Demographic and Clinical Characteristics Between Liver Injury and Non-Liver Injury Groups

Characteristics	Total (n=156)	Patients with Hepatotoxicity (n=52)	Patients without Hepatotoxicity (n=104)	Statistical value	*P*-value
Age (years), M (Q25, Q75)	65.00 (59.30, 70.00)	62.5 (57.0, 68.0)	66.0 (61.0, 71.8)	2042.5	0.013
Gender, n (%)				0.020	0.887
Male	125 (80.1%)	42 (80.8%)	83 (79.8%)		
Female	31 (19.9%)	10 (19.2%)	21 (20.2%)		
Hypertension, n (%)				2.373	0.123
Yes	55 (35.3%)	14 (26.9%)	41 (39.4%)		
No	101 (64.7%)	38 (73.1%)	63 (60.6%)		
Diabetes mellitus, n (%)				2.792	0.095
Yes	26 (16.7%)	5 (9.6%)	21 (20.2%)		
No	130 (83.3%)	47 (90.4%)	83 (79.8%)		
Smoking, n (%)				0.013	0.908
Yes	95 (60.9%)	32 (61.5%)	63 (60.6%)		
No	61 (39.1%)	20 (38.5%)	41 (39.4%)		
Alcohol drinking, n (%)				0.468	0.494
Yes	69 (44.2%)	21 (40.4%)	48 (46.2%)		
No	87 (55.8%)	31 (59.6%)	56 (53.8%)		

(*Continued*)

https://doi.org/10.2147/TCRM.S510973

Table 2 (Continued).

Characteristics	Total (n=156)	Patients with Hepatotoxicity (n=52)	Patients without Hepatotoxicity (n=104)	Statistical value	*P*-value
Combination chemotherapy, n (%)				1.130	0.288
Yes	138 (88.5%)	48 (92.3%)	90 (86.5%)		
No	18 (11.5%)	4 (7.7%)	14 (13.5%)		
Hepatic metastasis, n (%)				1.405	0.236
Yes	20 (12.8%)	9 (17.3%)	11 (10.6%)		
No	136 (87.2%)	43 (82.7%)	93 (89.4%)		
Concomitant medications, n (%)					
Statins	15 (9.6%)	5 (9.6%)	10 (9.6%)	0.000	1.000
PPIs	61 (39.1%)	21 (40.4%)	40 (38.5%)	0.054	0.817
Heparins	13 (8.3%)	7 (13.5%)	6 (5.8%)	1.773	0.183
Laboratory data at the baseline					
PT (S), M (Q25, Q75)	12.70 (12.10, 13.40)	12.90 (12.30, 13.60)	12.50 (12.00, 13.30)	2245.50	0.101
INR, M (Q25, Q75)	1.05 (1.00, 1.12)	1.06 (1.01, 1.12)	1.04 (1.00, 1.10)	2398.50	0.289
Fib (g/L), M (Q25, Q75)	3.73 (3.08, 4.43)	3.74 (3.04, 4.59)	3.73 (3.10, 4.42)	2527.50	0.568
TT (S), M (Q25, Q75)	15.90 (15.20, 16.70)	15.70 (15.30, 16.40)	16.00 (15.20, 16.80)	2364.00	0.234
APTT (S), M (Q25, Q75)	27.40 (25.20, 29.70)	27.90 (25.78, 29.60)	27.20 (24.80, 29.80)	2543.00	0.609
D-D (μg/mL), M (Q25, Q75)	0.97 (0.62, 1.87)	1.09 (0.70, 2.65)	0.91 (0.58, 1.48)	1353.00	0.140
WBC (10^9/L), M (Q25, Q75)	6.41 (4.93, 7.93)	6.24 (4.87, 7.94)	6.47 (5.08, 7.93)	2569.00	0.612
HGB (g/L), mean ± SD	118.70±1.70	118.50±20.74	118.80±21.49	−0.083	0.934
PLT (10^9/L), M (Q25, Q75)	239.00 (191.00, 322.25)	251.00 (183.75, 315.50)	238.00 (191.00, 326.25)	2693.00	0.967
GRAN (%), mean ± SD	65.96±0.90	66.38±11.83	65.76±11.02	0.324	0.747
TP (g/L), mean ± SD	64.96±0.51	65.01±6.37	64.93±6.44	0.069	0.945
GGT (U/L), M (Q25, Q75)	23.00 (16.25, 37.00)	34.50 (20.25, 56.00)	20.00 (15.00, 32.00)	1625.00	<0.001
ALT (U/L), M (Q25, Q75)	14.00 (10.00, 21.00)	20.50 (14.00, 30.00)	12.50 (9.00, 17.75)	1470.50	<0.001
AST (U/L), M (Q25, Q75)	17.00 (14.00, 22.00)	22.00 (17.00, 29.00)	16.00 (13.25, 19.00)	1379.00	<0.001
ALB (g/L), mean ± SD	38.56±0.40	39.12±5.26	38.29±4.85	0.983	0.327
DBil (umoL/L), M (Q25, Q75)	3.70 (2.70, 4.60)	3.70 (2.60, 4.98)	3.75 (2.70, 4.60)	2664.00	0.880
TBil (umoL/L), M (Q25, Q75)	10.75 (7.70, 13.80)	10.15 (7.63, 13.95)	10.90 (7.93, 13.70)	2684.50	0.942
ALP (U/L), M (Q25, Q75)	80.00 (65.25, 101.75)	78.50 (63.00, 105.75)	80.50 (67.00, 101.00)	2606.50	0.714
SCR (μmol/L), M (Q25, Q75)	57.00 (50.00, 68.00)	57.00 (51.00, 67.75)	57.50 (49.00, 68.75)	2651.50	0.843
BUN (mmol/L), M (Q25, Q75)	5.10 (4.13, 6.68)	4.95 (3.80, 6.95)	5.10 (4.20, 6.50)	2534.50	0.524
PD-1 inhibitors, n (%)					
Camrelizumab	38 (24.4%)	13 (25.0%)	25 (24.0%)	0.017	0.895
Tislelizumab	40 (25.6%)	14 (26.9%)	26 (25.0%)	0.067	0.795
Sintilimab	60 (38.5%)	19 (36.5%)	41 (39.4%)	0.122	0.727
Toripalimab	16 (10.3%)	5 (9.6%)	11 (10.6%)	0.035	0.852
Nivolumab	2 (1.3%)	1 (1.9%)	1 (1.0%)	0.000	1.000
Pre-existing liver disease, n (%)				1.693	0.710
Hepatic cyst	17 (10.9%)	6 (11.5%)	11 (10.6%)	0.033	0.856
Hepatic hemangioma	3 (1.9%)	0 (0%)	3 (2.9%)	0.382	0.536
Hepatitis B	2 (1.3%)	1 (1.9%)	1 (1.0%)	0.000	1.000
Primary tumour type, n (%)					
Lung cancer	47 (30.1%)	16 (30.8%)	31 (29.8%)	0.015	0.902
Gastric cancer	47 (30.1%)	15 (28.8%)	32 (30.8%)	0.061	0.805
Esophagus cancer	24 (15.4%)	7 (13.5%)	17 (16.3%)	0.222	0.638
Bladder cancer	10 (6.4%)	3 (5.8%)	7 (6.7%)	0.000	1.000
Others	28 (17.9%)	11 (21.2%)	17 (16.3%)	0.544	0.461
Tumor stage 4, n (%)	67 (42.9%)	24 (46.2%)	43 (41.3%)	0.327	0.567

Abbreviations: PPIs, proton pump inhibitors; ALT, alanine transaminase; AST, aspartate transaminase; DBil, direct bilirubin; TBil, total bilirubin; ALB, albumin; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TP, total protein; SCR, serum creatinine; BUN, blood urea nitrogen; TT, thrombin time; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio of prothrombin time; Fib, fibrinogen; D-D, D-Dimer; PLT, platelet; WBC, white blood cell; HGB, hemoglobin; GRAN, the percent of neutrophile granulocyte.

Table 3 Binary Logistic Regression Analysis of Risk Factors Associated with Liver Injury

Variables	β	SE	Wald χ^2	*P* value	OR	95% CI
GGT (U/L)	0.021	0.009	5.430	0.020	1.021	1.125–1.039
ALT (U/L)	0.034	0.027	1.593	0.207	1.035	0.981–1.091
AST (U/L)	0.118	0.043	7.584	0.006	1.125	1.035–1.224

Abbreviations: GGT, gamma-glutamyltransferase; ALT, alanine transaminase; AST, aspartate transaminase.

hypertension, diabetes mellitus, smoking, alcohol drinking, combination chemotherapy, hepatic metastasis, concomitant medications (statins, PPIs and heparins), type of PD-1 inhibitor and primary tumor, pre-existing liver disease (hepatic cyst, hepatic hemangioma and hepatitis B) and tumor stage, as well as some laboratory test results, such as DBil, TBil, ALB, ALP, TP, SCR, BUN, TT, PT, APTT, INR, Fib, D-D, PLT count, WBC count, HGB and GRAN.

Risk Factors of PD-1 Inhibitors Associated with Liver Injury

Using liver injury as the dependent variable, a multivariate logistic regression analysis was performed to identify risk factors for liver injury. Variables with statistically significant differences ($p<0.05$) in the univariate analysis, including age, GGT, ALT, and AST, were selected as independent variables. Results indicated that GGT (OR=1.021, 95% CI: 1.125–1.039, $p<0.05$) and AST (OR=1.125, 95% CI: 1.035–1.224, $p<0.01$) were independent risk factors associated with the occurrence of liver injury. The detailed results were presented in Table 3.

Furthermore, using liver injury as the outcome variable and predictors as test variables, we plotted the ROC curves. The results of the ROC curve analysis were showed in Figure 4. The closer the ROC curve is to the top-left corner, the more accurate the predictive ability of the model. Results showed that, the area under the curve (AUC) for GGT was 0.700 ($p<0.001$, 95% CI: 0.612–0.787), with a cutoff value of 28.5 U/L for predicting liver injury (sensitivity: 63.5%, specificity: 69.2%). The AUC for AST was 0.745 ($p<0.001$, 95% CI: 0.660–0.830), with an optimal cutoff value of 19.5 U/L (sensitivity: 65.4%, specificity: 77.9%). For ALT, the AUC was 0.728 ($p<0.001$, 95% CI: 0.640–0.816), with an optimal cutoff value of 19.5 U/L (sensitivity: 55.8%, specificity: 80.8%). Using the cutoff values mentioned above as

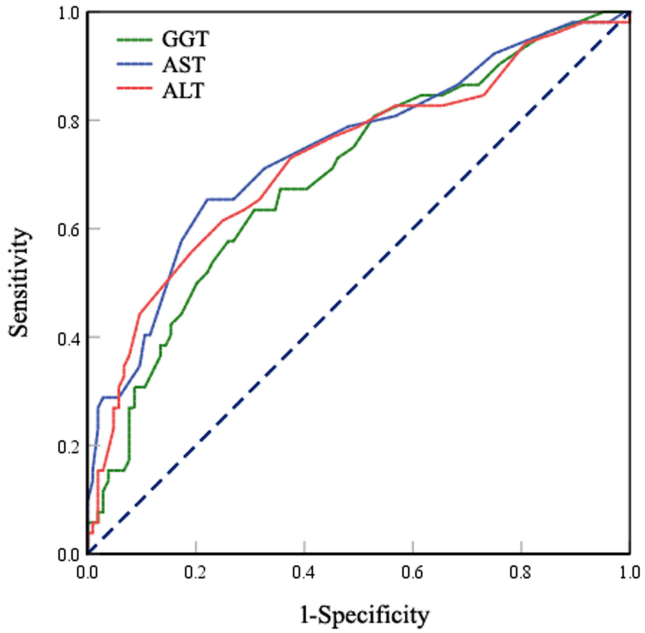

Figure 4 ROC curves of GGT, AST and ALT in predicting the risk of PD-1 inhibitors-induced liver injury.

https://doi.org/10.2147/TCRM.S510973

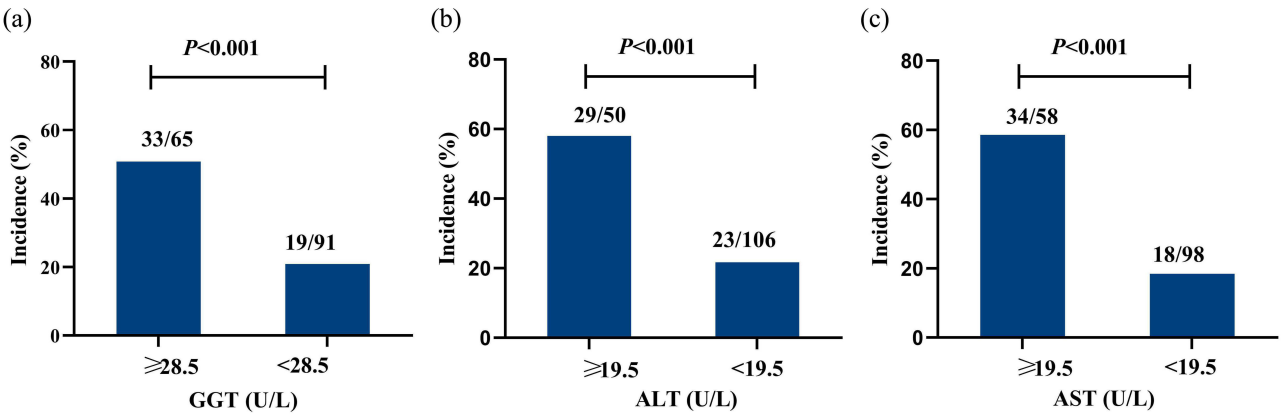

Figure 5 Comparison of the incidence of liver injury between groups based on the cutoff values: (**a**) GGT≥28.5 U/L and GGT<28.5 U/L group; (**b**) ALT≥19.5 U/L and ALT<19.5 U/L group; (**c**) AST≥19.5 U/L and AST<19.5 U/L group.

alert thresholds, patients were divided into two groups to compare the incidence of liver injury. In the patients with GGT≥28.5 U/L group, the incidence of liver injury was 50.8% (33/65), while in the GGT<28.5 U/L group, it was 20.9% (19/91). For ALT, the incidence was 58.0% (29/50) in the ALT≥19.5 U/L group and 21.7% (23/106) in the ALT<19.5 U/L group. For AST, the incidence was 58.6% (34/58) in the AST≥19.5 U/L group and 18.4% (18/98) in the AST<19.5 U/L group. The differences between the groups were all statistically significant (χ^2=15.244, p<0.001; χ^2=20.148, p<0.001; χ^2=26.567, p<0.001) (Figure 5).

Discussion

ICPIs-related liver injury has emerged as a new challenge in the field of drug-induced liver injury in recent years. Due to significant differences in its pathogenesis, clinical characteristics, and treatment options compared to previously reported cases of DILI, the European Association for the Study of the Liver (EASL) published clinical practice guidelines on drug-induced liver injury in 2019 highlighted ICPIs-related liver injury as a special type of DILI, primarily caused by enhanced immune responses, with a relatively high incidence and varying severity that can be difficult to predict.[10]

Liver injury caused by ICPIs treatment can manifest as asymptomatic elevations in transaminase levels in mild cases, while severe cases may lead to acute liver failure. Our study found that liver injury related to PD-1 inhibitors was predominantly mild. Patients typically presented with elevated ALT and/or AST, with or without elevated bilirubin, along with symptoms such as fatigue and loss of appetite, which was consistent with previous reports. ICPI-related liver injury can occur at any time after the first dose, most commonly between 4 and 12 weeks after treatment initiation, although there have also been case reports of occurrences nearly one year after starting therapy.[6,11] We found that the onset of liver injury in 52 patients ranged from 2 to 219 days, with 71.15% occurring within 30 days, suggesting that early monitoring of liver function was critical in the PD-1 inhibitor treatment, as timely intervention can prevent further deterioration of liver function. Studies have reported that ICPIs-induced liver injury may present as cholestatic, mixed-type, or hepatocellular injury, with PD-1/PD-L1 inhibitors more frequently associated with cholestatic or mixed-type liver injury compared to CTLA-4 inhibitors.[12,13] In this study, 48.0% of the liver injury cases were classified as cholestatic, 1.9% as hepatocellular, and 50.0% as mixed-type, which was consistent with previous findings. According to ESMO clinical practice guideline,[14] treatment with prednisone was recommended for patients with grade 2 or higher liver injury. If liver function does not improve after 3 days of steroid therapy, additional immunosuppressants such as tacrolimus or mycophenolate mofetil may be administered. Overall, the prognosis of ICPIs-related liver injury is relatively favorable, with most cases resolving spontaneously or improving with corticosteroid therapy. In our study, 39 out of 52 patients received hepatoprotective agents, corticosteroids, or a combination of both after the onset of liver injury, while 13 patients did not receive any medication. We found that 75% of the patients achieved full recovery of liver function, 5.8% showed partial recovery, and 13.5% experienced worsening liver injury.

The exact mechanisms underlying ICPIs-related liver injury remain unclear. Some studies suggested that the primary mechanism involved the blockage of key checkpoints in negative immune regulation by ICPIs, leading to an over-activation of the immune system and a loss of immune tolerance to self-tissues, which altered liver's immune microenvironment.[15,16] However, the specific pathogenesis is still under further investigation. The reported incidence of ICPIs-induced liver injury varies across different studies. A meta-analysis by Li et al[17] on the Chinese population found that the incidence of liver injury following treatment with pembrolizumab, nivolumab, camrelizumab, toripalimab, tislelizumab, and sintilimab ranged from 7.4% to 14.0%, with an incidence of 6.9% to 13.1% for ICPIs monotherapy. Another meta-analysis by Fu et al[18] reported that the incidence of PD-1/PD-L1 inhibitor-related liver injury was 1.24% (0.91–1.68%). In our study, active monitoring was conducted for five PD-1 inhibitors, with an overall liver injury incidence of 0.34%. Nivolumab had the highest incidence at 2.86%, followed by sintilimab at 0.41%, toripalimab and camrelizumab both at 0.34%, and tislelizumab at 0.28%. The four newly marketed PD-1 inhibitors demonstrated a similar incidence of liver injury and lower than previously reported studies, which may be due to the smaller sample size in this study.

Currently, research findings on the factors influencing ICPIs-related liver injury were inconsistent. Sawade et al[19] found that non-alcoholic fatty liver disease was an independent risk factor for liver injury related to PD-1 inhibitors (HR=29.34, $p<0.01$). Cho et al[20] reported that concomitant use of acetaminophen (HR=2.139, $p<0.05$) and statins (HR=4.706, $p<0.05$) were risk factors for liver injury when using immune checkpoint inhibitors, with male patients (HR=1.608, $p<0.05$) and those under 65 years of age (HR=1.527, $p<0.05$) experiencing liver injury more rapidly. A meta-analysis by Wang et al[21] on irAEs associated with PD-1/PD-L1 inhibitors revealed that among 12,808 patients, the incidence rates of elevated ALT, AST, TBil, and hepatitis were 4.2%, 4.4%, 2.0%, and 1.2%, respectively; the incidence rates of severe elevations in ALT, AST, TBil, and hepatitis were 1.9%, 1.8%, 1.3%, and 1.0%, respectively. In this study, we found that the elevated baseline GGT (OR=1.021, $p<0.05$) and elevated baseline AST (OR=1.125, $p<0.01$) were independent risk factors for liver injury in cancer patients. Additionally, results indicated that baseline ALT, baseline AST, and baseline GGT were all feasible predictive indicators for PD-1 inhibitor-related liver injury. We recommend strengthening medication monitoring for patients with baseline ALT≥19.5 U/L, baseline AST≥19.5 U/L, and baseline GGT≥28.5 U/L. Regular biochemical testing is advised to promptly identify liver function abnormalities and enable timely intervention.

This study had several limitations. Firstly, this study utilized a retrospective case-control design, which unavoidably incorporates certain biases and confounding variables. Secondly, although the study was conducted in a large general hospital, the findings may not be fully generalizable to broader patient populations. Multicenter, large-scale real-world studies are needed to further elucidate the incidence and risk factors associated with PD-1 inhibitor-related liver injury.

Conclusions

In conclusion, the incidence of liver injury among cancer patients treated with PD-1 inhibitors immunotherapy was 0.34%, with incidence of 0.41% for sintilimab, 0.34% for both toripalimab and camrelizumab, and 0.28% for tislelizumab. Overall, the mean time from the commencement of immunotherapy to the manifestation of liver injury was 28.4 days. Most patients (71.15%) developed liver injury within the first 30 days. The median age of the patients was 62.5 years, and patients over 50 years old accounted for 86.5%. Increased baseline GGT and AST levels were identified as independent risk factors for liver injury. Patients with high baseline levels of GGT, ALT, and AST were at increased risk of developing liver injury. We recommend that patients with baseline ALT≥19.5 U/L, AST≥19.5 U/L, and GGT≥28.5 U/L should receive enhanced monitoring of liver function indicators during PD-1 inhibitor treatment to prevent or mitigate the adverse events of liver injury, particularly in patients older than 50 years, and within the first 30 days after initiating PD-1 inhibitor treatment.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author.

Funding

This study was supported by Shandong Province Adverse Drug Reaction Monitoring Sentinel Research Project (No. 2023SDADRKY43).

Disclosure

The authors report no conflicts of interest in this work.

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