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Predicting the occurrence of liver injury induced by immune checkpoint inhibitors in hepatocellular carcinoma patients: a retrospective analysis

Xiaoyan Li^{1*†}, Hang Sun^{1,2†}, Jialiang Wang³ and Lubiao Chen^{1*}

Abstract

Purpose Immune checkpoint inhibitors (ICIs) have been widely used in the systemic treatment of hepatocellular carcinoma (HCC). Immune-mediated liver injury caused by ICIs (ILICI) often leads to anti-tumor therapy suspension or interruption and adversely affects prognosis. Therefore, this study retrospectively analyzed its characteristics, searched for its risk factors and attempted to establish a predictive model.

Methods A total of 207 inpatients with HCC who received ICIs treatment were retrospectively analyzed. They were divided into ILICI group ($n = 52$) and Non-ILICI group ($n = 155$) according to whether ILICI occurred or not, and the clinical characteristics of ILICI were analyzed. The risk factors were screened by logistic regression analysis between these two groups, and then a predictive model for ILICI occurrence was established.

Results Overall, ILICI mostly occurred on 4 to 12 weeks after ICIs treatment was initiated with an incidence 25.1%. The clinical types of liver injury were cholestasis (65.4%), hepatocellular (11.5%) and mixed (23.1%) respectively. The severity of ILICI mostly was mild with 40 cases (76.9%). Diabetes (odds ratio [OR] 3.026, 95% confidence interval [CI] 1.057–8.661, $P = 0.039$), cirrhosis (OR 6.758, 95% CI 1.433–31.882, $P = 0.016$) and multiple (3 or more) nodules of HCC (OR 3.097, 95% CI 1.532–6.261, $P = 0.002$) were risk factors for the occurrence of ILICI through multivariate analysis. A model was established to predict ILICI occurrence with the area under the receiver operating characteristic curve 0.701, sensitivity 0.712, specificity 0.632, positive predictive value 0.720 and negative predictive value 0.630.

Conclusions ILICI in HCC patients is not rare, especially in those with diabetes, liver cirrhosis or multi-nodular liver tumors. Although most cases are mild, it should not be overlooked. The model we established to predict ILICI occurrence has certain value and is helpful for clinicians to make clinical decisions.

Keywords Hepatocellular carcinoma, Immune checkpoint inhibitors, Immune liver injury caused by ICIs (ILICI), Risk factor, Predict

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Introduction

Hepatocellular carcinoma (HCC) is a major health concern worldwide, and ranks as the third leading cause of cancer related death globally [1, 2]. In China, the number of patients with HCC accounts for nearly half of the global liver cancer cases, making it one of the countries with the highest burden of HCC [3]. The treatment landscape of HCC is characterized by multidisciplinary participation and the coexistence of various therapeutic approaches. However, given the insidious onset, rapid progression and early tendency for metastasis in HCC, it frequently presents as an advanced disease at diagnosis. In addition, disease relapse following surgical treatments is frequent. Those make systemic therapy an important part of treatment [4]. In past years, particularly immunotherapies with immune checkpoint inhibitors (ICIs) have demonstrated impressive clinical survival benefits and become a milestone in the systematic treatment of advanced malignant tumors, including HCC [5–8].

Cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), programmed cell death protein-1 (PD-1) and its ligand (PD-L1) are the most common targeted immune checkpoints used in cancer immunotherapy. Numerous clinical studies targeting PD-1/PD-L1 and CTLA-4 pathways with ICIs have been conducted in the treatment of HCC in recent years [9, 10]. However, broadly enhancing immune effects can lead to side effects, known as immune-related adverse events (IrAEs), which become a barrier to tumor immunotherapy. The organs most commonly affected by IrAEs are the skin, liver, endocrine system and gastrointestinal tract [11, 12]. Although most phase III globally clinical trials in HCC have shown that ICIs generally have a good tolerability profile with the incidence of liver injury range from 3.6% to more than 10% [10, 13, 14], several retrospective studies have showed that the incidence and severity of ILICIs significantly increased in HCC systematic therapy than those in other malignant tumors [15–18]. Unlike other countries, the predominant cause of liver cancer in our country is chronic HBV infection and most of these HCC patients undergoing the “hepatitis-cirrhosis-liver cancer” progression, which distinguishes liver cancer from other tumor types [19–22]. ICIs combined with targeting anti-angiogenesis therapy [23–26] or/and local treatment [27, 28] have become a major treatment strategy in real world practice. Theoretically, all these made HCC patients more susceptible to develop liver injury during systematic therapy. Moreover, HCC patients underwent with ILICI had a poor prognosis [29]. Most of ILICI are mild and can resolve spontaneously, but there are also severe cases resulting in death [30]. Thus, we should pay high attention to the ILICI in HCC patients and find some predictive factors. Researches have demonstrated that some cytokines such as IL-6, CXCL9, CXCL10, CXCL11,

CXCL13, pre-existing conditions like non-alcoholic fatty liver disease, gender, history of ICIs treatment and the type of ICIs may be potential risk factors for ILICI [31–33]. However, most of these researches focus mainly on non-liver cancer and it remains uncertain whether these findings apply to ILICI in HCC patients. There is a lack of reliable biomarkers to predict the ILICI occurrence in HCC patients.

In this retrospective investigation, we summarized the clinical characteristics of ILICI, analyzed its potential risk factors and aimed to establish a model to predict ILICI occurrence.

Materials and methods

Study participants

We conducted a case search using the Hospital Information System (HIS) with the keywords “liver cancer,” “hepatocellular carcinoma,” “HCC” or “malignant liver tumor.” The search covered inpatient cases from September 1, 2019 to August 31, 2022 and yielded 3,556 cases. Among them, 3,058 cases did not receive ICIs treatment. After reviewing and screening the medical records, duplicate hospitalizations of the same patient were merged. A total of 498 patients received at least one dose of ICIs at our hospital. Of these, 291 patients had missing key baseline data or lacked follow-up data. The remaining 207 patients met the requirements of this study protocol. Among these, 155 cases did not experience ILICI, while 52 cases experienced varying degrees of ILICI.

Inclusion criteria for this study were summarized as follows: (1) Patients had a radiological diagnosis of HCC according to the American Association for the Study of Liver Diseases (AASLD) criteria [34]; (2) Received at least one dose of ICIs treatment at our hospital; (3) Underwent at least one follow-up liver function test after ICIs treatment; (4) Had relatively complete baseline data before ICIs treatment. Exclusion criteria were as follows: (1) Missing key data (baseline data before ICIs treatment or follow-up data after treatment); (2) Patients whose initial ICIs treatment was not conducted at our hospital (Fig. 1). The study was approved by the Institutional Review Board of the Sun Yat-sen University (No. [2022] 02-294-01). The study was retrospective and the requirement for informed consent was therefore waived.

Demographic information, laboratory test parameters and tumor treatment

We collected baseline demographic information and laboratory test parameters for all patients, including age, gender, ethnicity, and underlying liver diseases, such as hepatitis B, hepatitis C, alcohol consumption, non-alcoholic fatty liver disease and their antiviral treatment before therapy. We also gathered data on whether patients had diabetes, autoimmune diseases or other

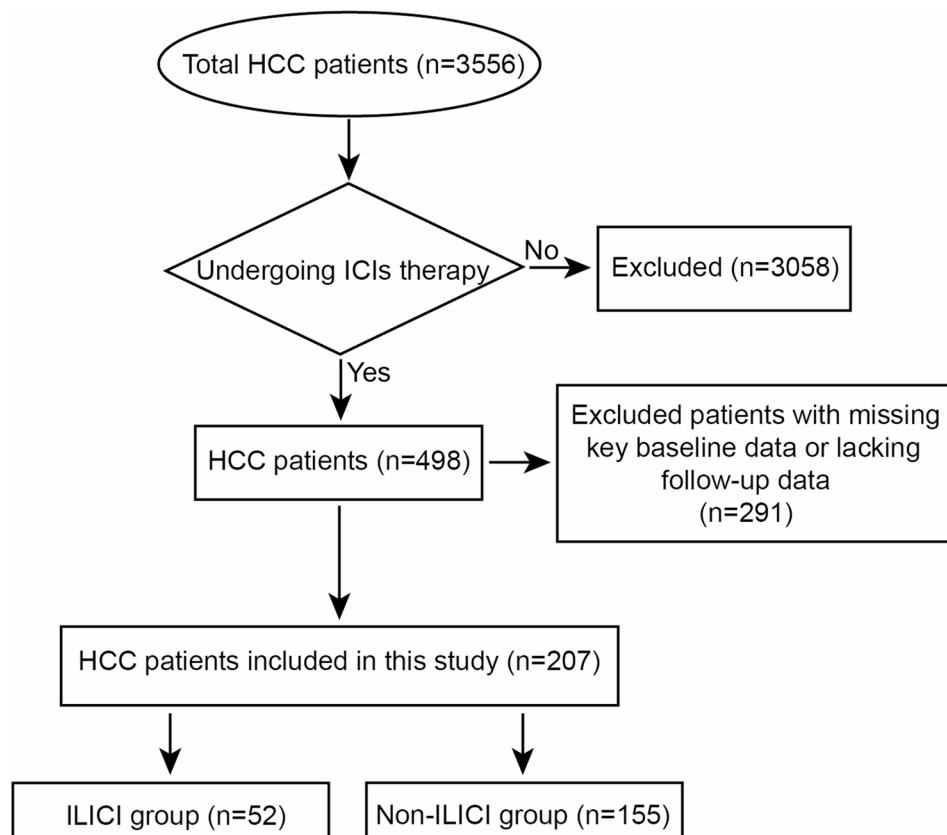


Fig. 1 Flow diagram of the screening and selection process

underlying conditions. Baseline laboratory tests included blood routine, liver function biochemistry, coagulation function and liver imaging characteristics. Tumor characteristics, such as alpha-fetoprotein (AFP) levels, tumor size, nodule number, macrovascular invasion, distant metastasis and Barcelona Clinic Liver Cancer (BCLC) stage, were also recorded. Additionally, we collected information on the patients' cancer treatment, including whether they received ICIs monotherapy, combined with anti-angiogenic targeted therapy and/or underwent local liver tumor treatments, such as ablation therapy, vascular interventional treatments.

Diagnosis criteria and definitions of academic terms

The diagnosis of HCC is based on clinical manifestations, laboratory tests and medical imaging such as color doppler ultrasound (US), contrast-enhanced ultrasound (CEUS), computed tomography (CT), magnetic resonance imaging (MRI) or pathological tissue/cytological examinations, which is in accordance with the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer (2022 edition). The BCLC staging system is used for HCC staging [35]. The assessment of underlying liver cirrhosis is based on imaging criteria [36] or non-invasive index including the aspartate aminotransferase (AST) to

platelet (PLT) Ratio Index (APRI) and Fibrosis-4 Index (FIB-4) [37]. Cirrhosis is presented if APRI score > 2 in adults or FIB-4 > 3.25. In this study, we defined long-term alcohol consumption as an alcohol intake exceeding 20 g/d for more than 5 years. Diabetes diagnosis criteria are consistent with the American Diabetes Association guideline [38].

Identification and grading/typing standards for ILICI

Patients experiencing hepatic adverse events underwent examinations to rule out elevations in alanine aminotransferase (ALT), AST, alkaline phosphatase (ALP) and total bilirubin (TBil) caused by other reasons. The diagnosis of ILICI was made through the comprehensive judgment of clinical physicians. The severity of ILICI was graded into 5 levels (G1-5) according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 criteria issued by the National Institutes of Health. When the patient's baseline biochemical indicators are normal, the grading standard is based on the ratio of the value after liver damage occurs to the upper limit of the normal value. When the patient's baseline biochemical indicators are abnormal, the grading standard is based on the ratio of the value after liver damage occurs to the baseline value before treatment. Additionally, the R value ((ALT/

upper limit of normal [ULN] of ALT)/(ALP/ULN of ALP ratio) is used for typing the pattern of liver injury.

Statistical analysis

This study employed SPSS 24.0 statistical software for data processing and analysis. Continuous variables were expressed as the mean $\pm$ standard deviation ($M \pm SD$) or the median (Md) with interquartile ranges (IQR). The independent samples t-test was used for normally distributed data, while the Mann-Whitney U rank-sum test was utilized for non-normally distributed medians. Categorical variables were represented as counts (rates) and comparisons of rates were made using the chi-square test or Fisher's exact probability method. Univariate logistic regression analysis was conducted to screen for risk factors associated with the occurrence of ILICI, and variables with a P value less than 0.05 were included in the multivariate logistic regression analysis. A predictive model equation for the occurrence of ILICI was established based on the results of the multivariate analysis. The predict values for each ILICI case were calculated using the equation. Discrimination of ILICI occurrence was evaluated by the receiver operating characteristic (ROC) curve. The cut-off value for predicting ILICI occurrence was generated using the Youden index method. The predictive efficiency of the model was

evaluated using the area under the ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The ROC curve was plotted using GraphPad 9.0 software.

Results

Baseline demographic information, underlying liver disease and comorbid chronic conditions of the patients

A total of 207 patients were enrolled in this study and categorized into the ILICI group (52, 25.1%) and the Non-ILICI group (155, 74.9%) based on whether they developed ILICI. Among all 207 patients, the majority were male (190, 91.8%) and the proportion of males in the Non-ILICI group was lower than that in the ILICI group (89.7% vs. 98.1%, $P=0.056$). Hepatitis B virus (HBV)-related HCC accounted for 198 cases (95.7%), while Hepatitis C virus (HCV)-related HCC accounted for 2 cases (1.0%). Both HCV-related HCC patients had undergone direct-acting antiviral (DAA) therapy for HCV, and HCV RNA was undetectable prior to ICIs therapy. Patients with long-term alcohol intake numbered 11 (5.3%). There were 7 patients (3.4%) with no underlying HBV/HCV/alcoholic liver disease associated with HCC. Among all HBV-related HCC patients, 8 cases had a history of long-term alcohol intake. Among the 2 HCV-related HCC patients, 1 case had a history of long-term alcohol intake. A total of 21 patients (10.1%) had concurrent diabetes, and 19 patients (9.2%) had concomitant hypertension. 179 patients (86.5%) showed signs of cirrhosis on liver imaging. APRI and FIB-4 suggested that 46 patients (22.2%) and 100 patients (48.3%) might have cirrhosis, respectively. The majority of patients had a Child-Turcotte-Pugh (CTP) class A classification, with only 1 patient having a baseline CTP score of C. This patient had hypoalbuminemia and ascites before ICIs therapy but without significant hyperbilirubinemia and coagulopathy. Despite a CTP score of C, the patient underwent ICIs treatment after a comprehensive evaluation by the clinical team taking into account the individual's circumstances and with the patient's consent (Table 1).

Laboratory test results including complete blood count and liver function test at baseline

Subsequently, we further explored the differences in common laboratory test results between ILICI group and Non-ILICI group. We found that there were no statistically significant differences in the complete blood count between ILICI group and Non-ILICI group, including white blood cell count (WBC), PLT, percentage of neutrophils (NEUT%), percentage of lymphocytes (LYMPH%), percentage of monocytes (MONO%), neutrophil-to-lymphocyte ratio (NLR), and monocyte-to-lymphocyte ratio (MLR), with their means or medians generally within the normal range. Similarly, there

Table 1 Baseline demographic information, underlying liver disease and comorbid chronic conditions of the patients

	Non-ILICI (n = 155)	ILICI (n = 52)	P value
Age(years)	52.32 $\pm$ 11.78	54.25 $\pm$ 9.93	0.287
Gender			0.056
Male	139(89.7%)	51(98.1%)	
Female	16(10.3%)	1(1.9%)	
History of viral hepatitis			0.172
HBV	150(96.8%)	48(92.3%)	
HCV	0(0%)	2(100%)	
Long-term alcohol consumption ¹	5(3.2%)	6(11.5%)	0.021
Cirrhosis ²			0.018
Medical imaging	129(83.2%)	50(96.2%)	
APRI>2	30(11.7%)	16(30.8%)	0.087
FIB-4>3.25	74(47.7%)	26(50.0%)	0.778
CTP			0.232
A	133(85.8%)	40(76.9%)	
B	22(14.2%)	11(21.2%)	
C	0(0%)	1(1.9%)	
Diabetes	11(7.1%)	10(19.2%)	0.012

Continuous variables are expressed as mean $\pm$ standard deviation ($M \pm SD$) or median (Md) with interquartile range. Categorical variables are presented as number (%)

HBV Hepatitis B virus, HCV Hepatitis C virus, APRI Aminotransferase (AST) to platelet (PLT) Ratio Index, FIB-4 Fibrosis-4 Index, CTP Child-Turcotte-Pugh

¹Alcohol consumption is defined as > 20 g/day with a drinking history of more than 5 years

²The presence of cirrhosis is based on medical imaging or APRI > 2 or FIB-4 > 3.25

were no significant statistical differences in the baseline liver function test of the two groups, including ALT, AST, TBil, gamma-glutamyl transferase (GGT), ALP, cholinesterase (CHE), albumin (ALB), globulin (GLB), and ALB-to-GLB ratio (A/G), as well as in coagulation functions, including prothrombin time (PT) and international normalized ratio (INR). Notably, the means or medians of baseline AST (54, 36–93), ALP (156.8±97.8) and GGT (178.3±140.0) were all higher than the normal range, which may be related to the fact that all included patients were HCC patients (Table 2).

Baseline tumor burden of patients

In current domestic and international guidelines, ICIs therapy is primarily recommended for advanced HCC (aHCC) or unresectable HCC (uHCC). This study also included a subset of early-stage HCC patients who received curative treatment sequentially combined with immunotherapy at our institution (5 cases at stage 0, 25 cases at stage A) as so-called adjuvant therapy to reduced postoperative tumor relapse. As mentioned previously, one patient with a CTP score of C due to hypoalbuminemia and ascites at stage D was deemed eligible to receive PD-1 monotherapy after comprehensive assessment by the clinical team. There were no statistically significant differences in tumor diameter sum, macrovascular invasion, distant metastasis, AFP levels, and BCLC stage between the two groups of patients. However, there was a statistical difference in the presence of multiple tumor nodules (≥ 3 nodules: 43.9% vs. 71.2%, $P < 0.001$) (Table 3).

Tumor treatment of patients

The main ICIs included in this study were PD-1 and PD-L1 monoclonal antibodies (mAb). The types and usage of PD-1 monoclonal antibodies are as follows:

Table 2 Laboratory test results of patients at baseline

	Non-ILICI (n = 155)	ILICI (n = 52)	P value
Complete blood count			
WBC($10^9/L$)	6.42 ± 2.54	6.90 ± 2.77	0.257
PLT($10^9/L$)	172 ± 83	173 ± 97	0.759
N%	66.0 ± 11.8	66.3 ± 11.3	0.870
L%	22.2 ± 13.6	21.6 ± 10.1	0.754
NLR	3.4(2.2, 5.2)	3.4(2.0, 4.9)	0.797
MLR	0.5(0.4, 0.7)	0.5(0.3, 0.7)	0.395
Liver function test			
ALT(U/L)	40(27, 59)	35(25, 51)	0.331
AST(U/L)	55(37, 96)	43(33, 86)	0.054
ALB(g/L)	36.0 ± 5.24	38.1 ± 5.24	0.110
GLB(g/L)	31.5 ± 6.2	31.2 ± 5.9	0.766
A/G	1.19 ± 0.31	1.27 ± 0.31	0.137
TBil(umol/L)	14.7(9.6, 23.1)	13.8(8.4, 20.5)	0.341
ALP(U/L)	159.7 ± 99.8	148.4 ± 91.3	0.471
GGT(U/L)	176.0 ± 128.9	185.6 ± 169.7	0.665
CHE(U/L)	4744 ± 2009	5116 ± 1828	0.239
PT(s)	14.2 ± 1.5	14.4 ± 1.1	0.340
INR	1.1(1.0, 1.2)	1.1(1.0, 1.2)	0.519

Continuous variables are expressed as mean ± standard deviation (M ± SD) or median (Md) with interquartile range

Nivolumab 240 mg, Pembrolizumab 200 mg, Camrelizumab 200 mg, Sintilimab 200 mg, Tislelizumab 200 mg, or Toripalimab 240 mg, administered intravenously every 3 weeks. The PD-L1 mAb used was Atezolizumab 1200 mg, administered intravenously every 3 weeks. A total of 196 patients (94.7%) received PD-1 mAb treatment, including Nivolumab in 4 cases (1.90%), Pembrolizumab in 7 cases (3.40%), Camrelizumab in 84 cases (40.56%), Sintilimab in 74 cases (35.76%), Tislelizumab in 19 cases (9.19%), and Toripalimab in 8 cases (3.90%). Eleven patients (5.29%) received PD-L1 mAb treatment,

Table 3 Baseline tumor burden of patients

	Non-ILICI (n = 155)	ILICI (n = 52)	P value
BCLC stage			0.659
0	3(1.9%)	2(3.8%)	
A	18(11.6%)	7(13.5%)	
B	16(10.3%)	5(9.6%)	
C	118(76.1%)	37(71.2%)	
D	0(0%)	1(1.9%)	
Tumor invasion/metastasis			
Macrovascular invasion	115(74.2%)	34(65.4%)	0.221
Distant metastasis	23(14.9%)	12(23.1%)	0.176
Macrovascular invasion and distant metastasis	21(13.5%)	8(15.4%)	0.743
Tumor Burden			
Multiple nodules (≥ 3)	68(43.9%)	37(71.2%)	< 0.001
Tumor diameter sum (cm)	11(6.6,14)	9.6(6.3,14)	0.649
AFP(> 20 mg/L)	119(76.8%)	41(78.8%)	0.758

Categorical variables are presented as number (%). Continuous variables are expressed as mean ± standard deviation (M ± SD) or median (Md) with interquartile range

all of whom were treated with the Atezolizumab plus Bevacizumab treatment regimen (referred to as the “T + A regimen”) (Figure S1). No patient received a combination treatment of two or more immune therapy drugs. A total of 134 patients (64.7%) received tyrosine kinase inhibitor (TKI) targeted therapy in conjunction with immunotherapy, including Sorafenib, Regorafenib, Apatinib, Anlotinib, Donafenib or Lenvatinib. A total of 158 patients (76.3%) underwent local treatment, including surgical resection, local ablation, and vascular interventional treatments (TACE/HAIC), with some patients receiving multiple types of local treatments. There were no significant statistical differences in treatment with PD-1 mAb, PD-L1 mAb, TKIs, Bevacizumab, or local treatments between ILICI group and Non-ILICI group (Table 4).

Characteristics of patients with ILICI

Upon further analysis of the ILICI group patients, we found that the median latent period for ILICI onset was the 6th week after ICIs therapy (Fig. 2A and B), and it mostly occurred after the second ICIs therapy cycle (Fig. 2C and D). We then divided the ILICI group into a mild liver injury group (G1-2) and a severe liver injury group (G3-5) according to the CTCAE v5.0 criteria. We observed that the baseline age of patients in the severe liver injury group (60 ± 13 years) was older than that of those in the mild liver injury group (53 ± 9 years) with a statistically significant difference ($P < 0.05$). The rate of hypertension between these two groups differed significantly. However, as the samples of ILICI patients with hypertension is only seven, it might not accurately reflect the real situation. No significant differences were found in other demographic and chronic disease conditions, laboratory tests, tumor burden at baseline and the latent period of ILICI occurrence (Table 5). We also analyzed the changes in HBV DNA levels before and after the onset of ILICI in HBV-related HCC patients. Comparing the pre-treatment HBV DNA levels in ILICI groups with HBV-related HCC, we found that the post-treatment HBV DNA levels were significantly lower (Fig. 3A).

Table 4 Tumor treatment of patients

	Non-ILICI (n = 155)	ILICI (n = 52)	P value
ICIs therapy			0.208
PD-1 mAb	145(93.5%)	51(98.1%)	
PD-L1 mAb	10(6.5%)	1(1.9%)	
Targeted therapy	104(67.1%)	38(73.1%)	0.421
TKI	96(61.9%)	38(73.1%)	0.146
Bevacizumab	20(12.9%)	2(3.8%)	0.067
Local treatment	123(79.4%)	35(67.3%)	0.077

Categorical variables are presented as number (%)

ICIs Immune checkpoint inhibitors, TKI Tyrosine kinase inhibitor

Among these ILICI group patients, mild liver injury group showed a significant decrease in HBV DNA compared to baseline (Fig. 3B), while the severe liver injury group also showed a downward trend, but without statistical significance (Fig. 3C). These results are likely related to the fact that all HBV-related HCC patients had initiated nucleos(t)ide analog antiviral therapy before starting immunotherapy. Among 52 cases of ILICI group, we further examined the grading, typing and subsequent treatment of liver injury. According to the CTCAE v5.0 grading criteria, there were 25 (48.1%) cases of G1, 16 (30.8%) cases of G2, 4 (7.7%) cases of G3, 6 (11.5%) cases of G4 and 1 (2.0%) case of G5. In this study, all 25 ILICI patients with G1 continued immunotherapy and their liver function recovered after using general hepatoprotective treatments. For patients with G2/3/4 ILICI, the majority showed improvement in liver function after discontinuing immunotherapy and receiving glucocorticoids (GC) monotherapy or mycophenolate mofetil (MMF) combination therapy. One patient ultimately died during hospitalization, who experienced fever, fatigue and jaundice, and was considered to have concurrent immune-related cardiac injury. Laboratory tests indicated a continuous deterioration in coagulation function and they were irreversible despite treatment with GC, MMF and immunoglobulins, but eventually succumbed to severe infection and died after 2 weeks of the first ICIs treatment (Fig. 4A). Further, using the R value typing criteria, we classified ILICI patients into hepatocellular injury type (6, 11.50%), cholestasis type (34, 65.40%) and mixed type (12, 23.10%) (Fig. 4B). There was no significant difference in the severity of liver injury among these different types of ILICI patients (Figure S2).

Risk factors for ILICI

The results have indicated that the incidence of ILICI is relatively high among patients with HCC treated with ICIs. Therefore, there is an urgent need to identify high-risk factors that can predict the occurrence of ILICI. From a total of 38 parameters in Tables 1, 2, 3 and 4, we selected 9 parameters with $P < 0.10$ for univariate binary logistic regression analysis. We then further selected 4 parameters with $P < 0.05$ from the univariate analysis for the following multivariate binary logistic regression analysis (Table 6). Based on the results of the multivariate analysis, we ultimately established a predictive model and obtained a predictive mathematical equation for the occurrence of ILICI: $\text{Ln}(\pi/1-\pi) = -3.734 + 1.223 \times X_1 + 2.003 \times X_2 + 1.157 \times X_3$. In the equation, π represents the probability of ILICI occurrence. Variables X_1 , X_2 and X_3 represent diabetes, liver cirrhosis and three or more liver cancer nodules, respectively. Their values of 1 or 0 mean with or without above situations. We further evaluated the predictive performance of the model by ROC.

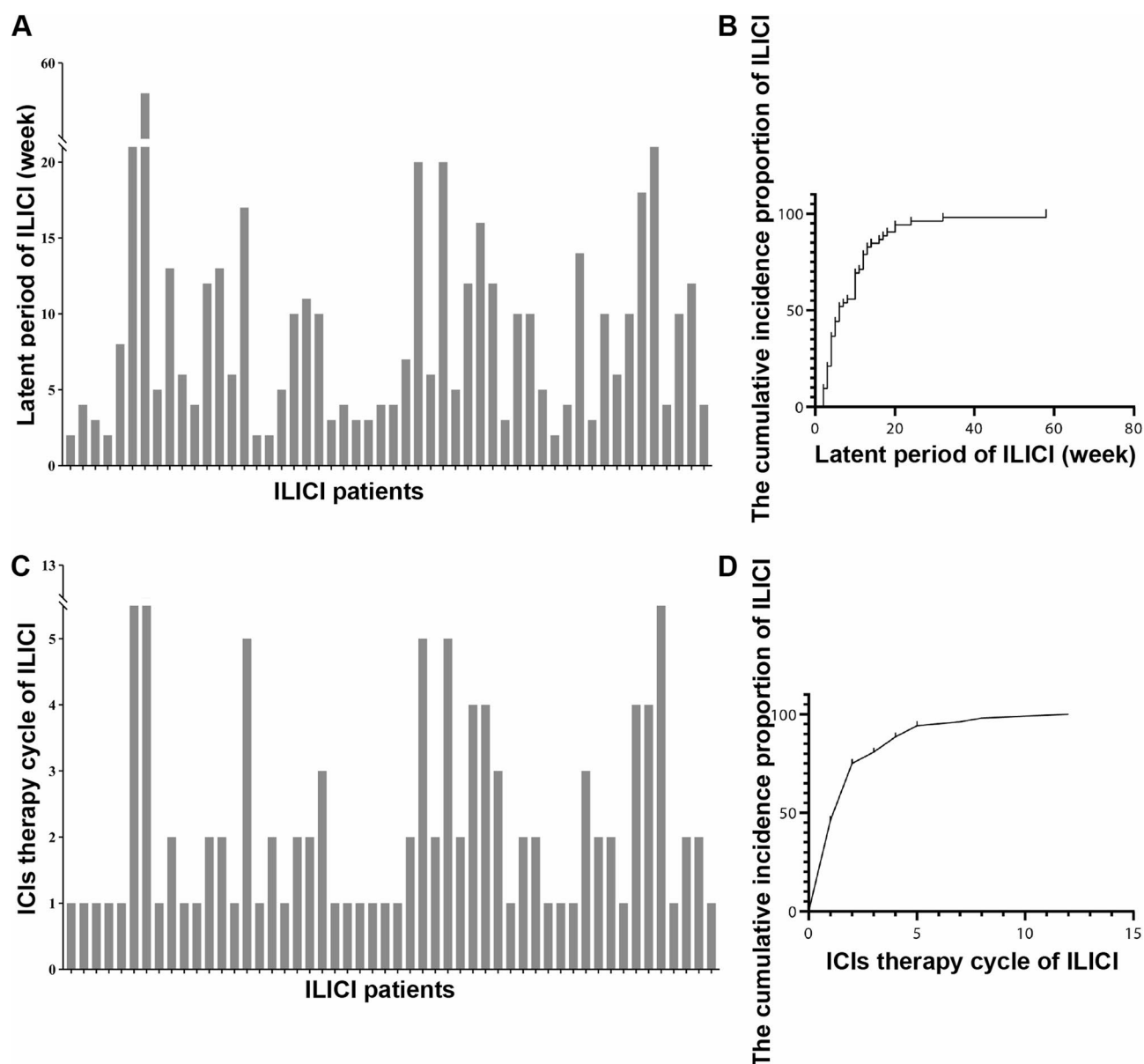


Fig. 2 The timing of ILICI occurrence after ICIs therapy. The median latent period for ILICI onset for each HCC patients with ILICI showed by bar plot **A** and cumulative incidence curves **B**. ICIs therapy cycle occurring ILICI for each HCC patients showed by bar plot **C** and cumulative incidence curves **D**

Our results revealed that the AUC was 0.701. The cut-off value (π) for predicting ILICI occurrence generated using the Youden index method was 0.283. The sensitivity, specificity, positive predictive value and negative predictive value were 0.712, 0.632, 0.72 and 0.63, respectively (Fig. 5).

Discussion

Previous reports on ILICI have largely focused on solid tumors such as melanoma, lung cancer, renal cell carcinoma and few on HCC. This study is a single-center, retrospective real-world study and primarily analyzes the clinical characteristics of ILICI in HCC patients. It also

attempts to identify risk factors for ILICI and constructs a predictive equation model. These findings provide new information for predicting the occurrence of ILICI.

With the widespread application of ICIs, their associated adverse reactions have increasingly gained the attention of clinical physicians. Researches have indicated that the overall incidence of liver injury was as high as 38%, and the incidence of G3 or higher liver injury was as high as 14.4% in various tumor types treated with ICIs. Moreover, the incidence of ILICI in HCC patients may be slightly higher than that in other tumors [15, 16, 39, 40]. In our cohort of 207 patients, ILICI occurred in 52 cases, with an incidence rate of 25.1%. Most of ILICI can resolve

Table 5 Differences between mild and severe group patients with ILICI

	Mild group (n=41)	Severe group (n=11)	P value
Age(years)	53±9	60±13	0.045
Gender			0.601
Male	40(97.6%)	11(100.0%)	
Female	1(2.4%)	0(0.0%)	
Long-term alcohol consumption ¹	4(9.8%)	2(18.2%)	0.437
Cirrhosis ²			
Medical imaging	39(95.1%)	11(100.0%)	0.455
APRI>2	12(29.3%)	4(36.4%)	0.651
FIB-4>3.25	20(48.8%)	6(54.5%)	0.734
Diabetes	8(19.5%)	2(18.2%)	0.921
ALP(U/L)	132±53	201±162	0.054
GGT(U/L)	166±139	259±249	0.107
Multiple nodules (≥ 3)	29(70.7%)	8(72.7%)	0.897
Latent period(week)	6(4,12)	5(3,13)	0.589

Continuous variables are expressed as mean±standard deviation (M±SD) or median (Md) with interquartile range. Categorical variables are presented as number (%)

APRI Aminotransferase (AST) to platelet (PLT) Ratio Index, FIB-4 Fibrosis-4 Index, ALP Alkaline phosphatase, GGT Gamma-glutamyl transferase

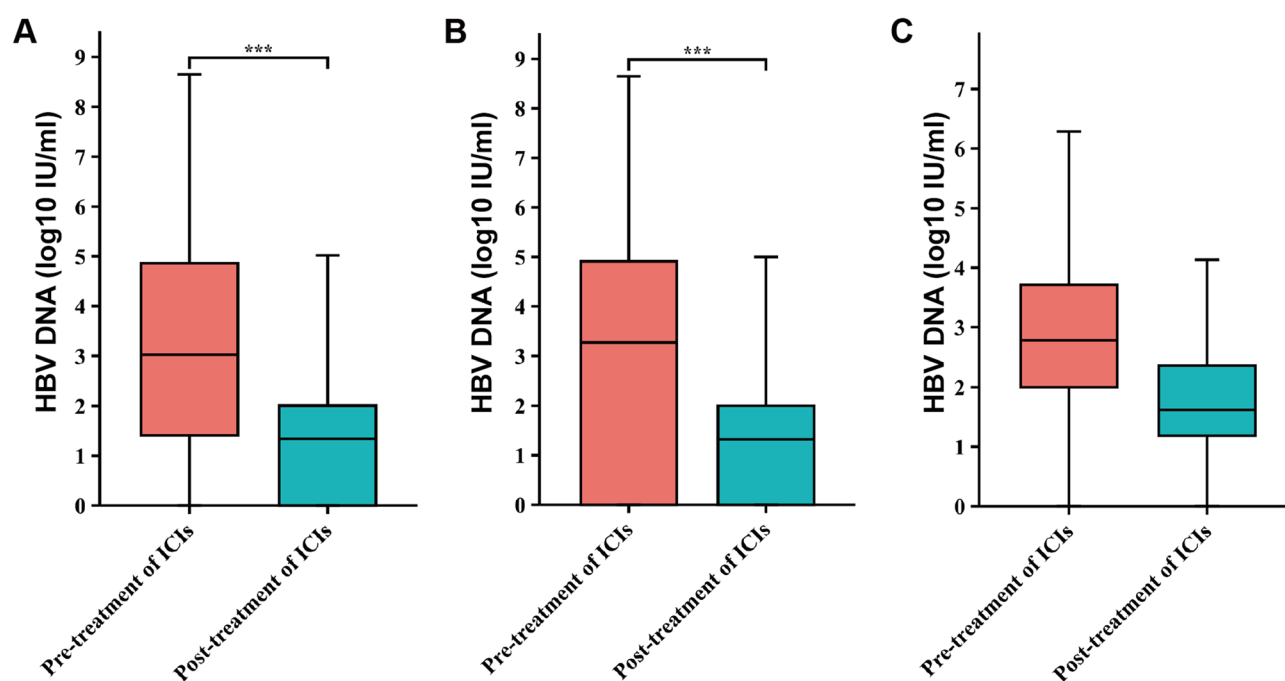


Fig. 3 The changes in HBV DNA levels before and after the onset of ILICI in HBV-related HCC patients. **A** Comparing the pre-treatment HBV DNA levels in ILICI groups with HBV-related HCC, the post-treatment HBV DNA levels of all HCC patients were significantly lower. **B** The mild ILICI group showed a statistically significant decrease in the post-treatment HBV DNA levels compared to the pre-treatment HBV DNA levels. **C** The severe ILICI group showed a downward trend without statistical significance in the post-treatment HBV DNA levels compared to the pre-treatment HBV DNA levels

spontaneously, but there are also severe cases resulting in death [30]. In our study, severe ILICI (G3 or higher) was observed in 11 cases, accounting for 21.2%. The onset of ILICI was predominantly between weeks 4 to 12, with a median time of 6 weeks, which is consistent with previous investigative studies [41]. The majority of patients developed hepatic injury after the second treatment cycle of ICIs and their clinical manifestations lacked distinct

specificity. The primary laboratory findings were elevated levels of ALT and AST, with some patients also showing increased levels of TBil, GGT and ALP which were indicative of cholestasis. In this study, the majority of ILICI patients showed a biochemical pattern of cholestasis (34, 65.4%). However, this classification method may not be entirely applicable to HCC patients, as the baseline levels of ALP/GGT in most patients in this study were already

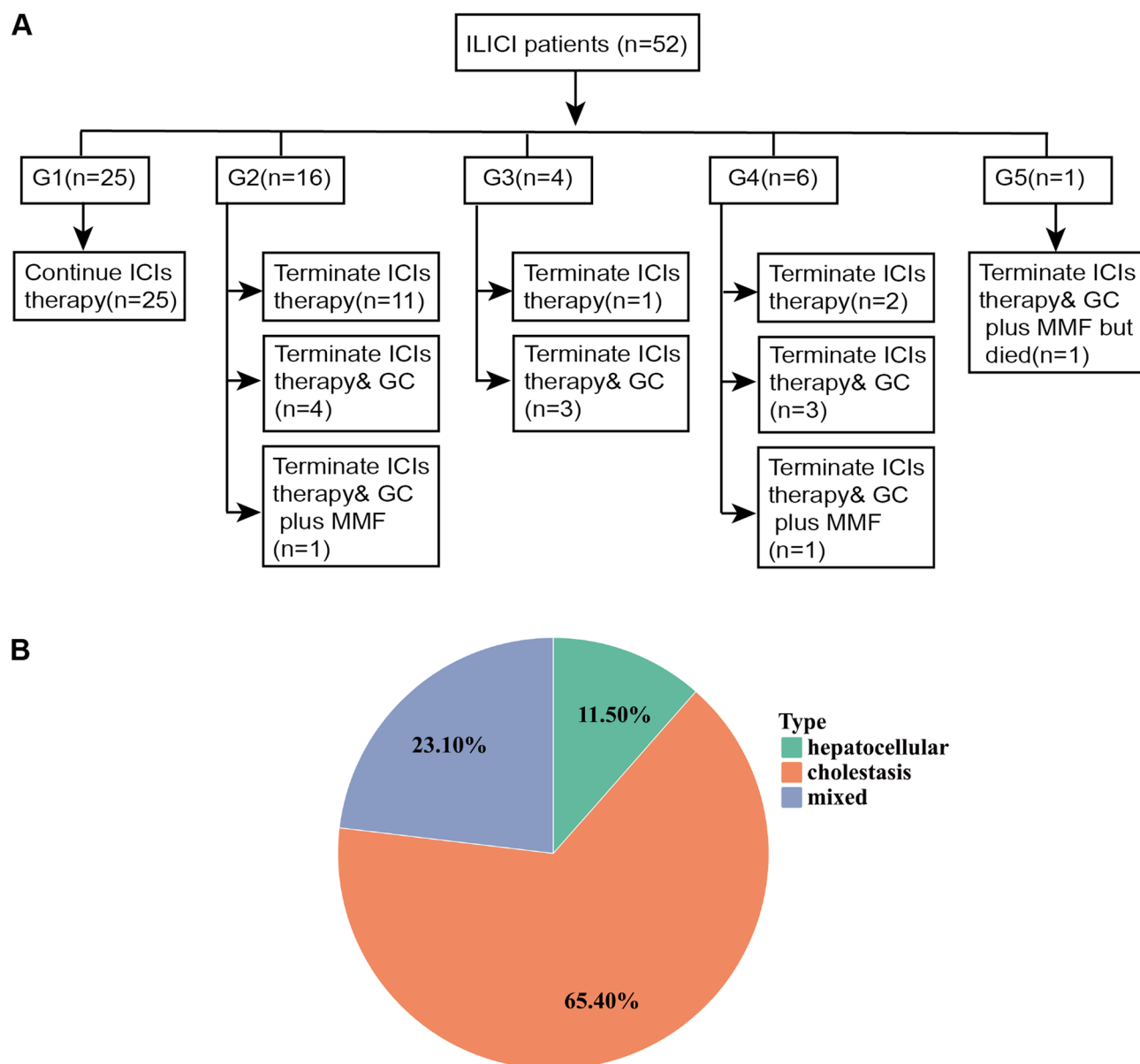


Fig. 4 Analysis of the grading, typing and subsequent treatment of liver injury among 52 cases of ILICI group. **A** The grading according to the CTCAE v5.0 criteria and their subsequent treatment of liver injury among 52 cases of ILICI group. **B** The typing among 52 cases of ILICI group

Table 6 Risk factors for ILICI

Factors	Univariate logistic regression analysis OR(95%CI)	P value	Multivariate logistic regression analysis OR (95%CI)	P value
Gender	0.170 (0.022,1.317)	0.090		
Long-term alcohol consumption	3.913 (1.142,13.413)	0.030	2.082 (0.548,7.916)	0.282
Cirrhosis by medical imaging	5.039(1.153,22.021)	0.032	6.758 (1.433,31.882)	0.016
Cirrhosis by APRI	1.013 (0.927,1.106)	0.780		
Diabetes	3.117 (1.239,7.843)	0.016	3.026 (1.057,8.661)	0.039
AST(U/L)	0.993 (0.986,1.000)	0.057		
Multiple nodules (≥ 3)	3.156 (1.601,6.221)	0.001	3.097 (1.532,6.261)	0.002
Bevacizumab	3.704(0.835,16.423)	0.085		
Local treatment	0.536 (0.267,1.076)	0.080		

CI Confidence interval, OR Odds ratio

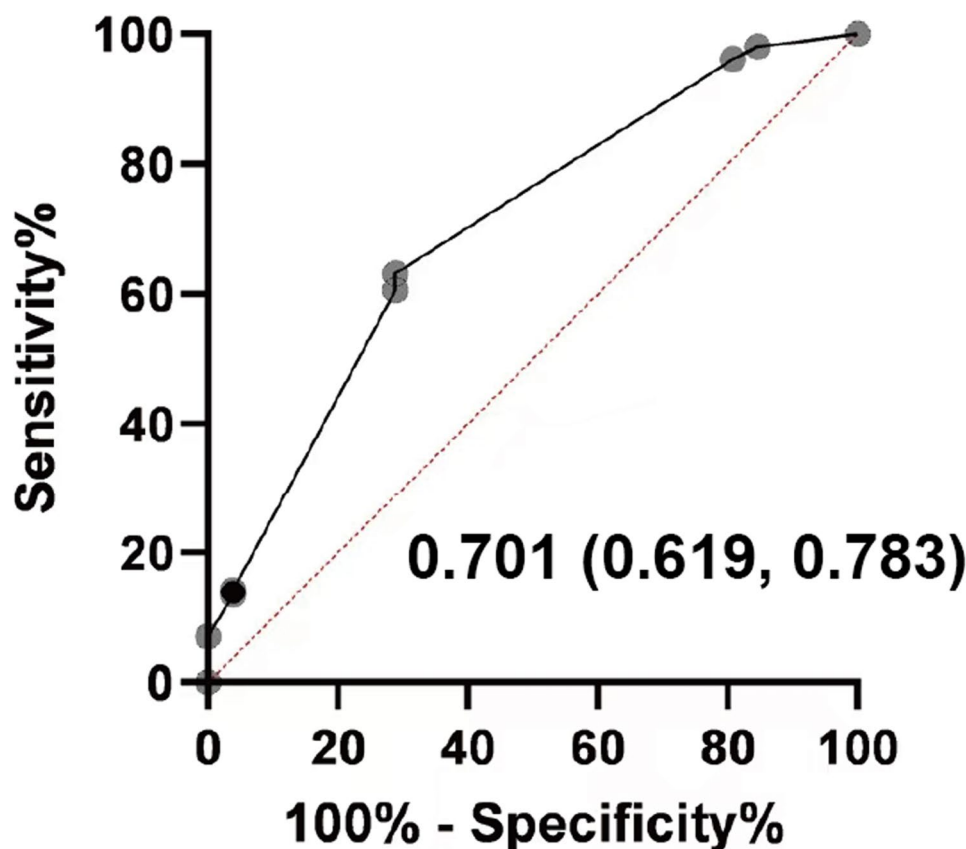


Fig. 5 ROC analysis for the prediction model of ILICI occurrence. ROC, receiver operator characteristic

higher than the ULN values, which is a biochemical characteristic inherent to HCC patients. Moreover, in this study, all the patients included were inpatients. However, some patients with G1-2 liver damage might have been overlooked or received outpatient treatment without being included in the data collection. This could potentially lead to bias in its classification. The classification criteria for type are primarily used for drug-induced liver injury and future research should aim to develop better classification methods.

Through the analysis of baseline clinical data, we found that the average age of patients with mild (G1/2) liver injury was lower significantly than that of patients with severe (G3/4) liver injury. This may suggest that older patients are more likely to experience severe liver injury. Age as a risk factor has also been observed in patients with other tumor types, but the findings are inconsistent [42, 43]. More studies are needed to focus on the role of age in ILICI in the future. In this study, the majority of patients had HBV-related HCC (198, 95.7%). Our study revealed that there was no statistically significant difference in baseline HBV DNA levels between the ILICI and Non-ILICI groups. This finding may be attributed to the fact that all HBV-related HCC patients had initiated antiviral therapy prior to the commencement of

immunotherapy and resulted in baseline HBV DNA levels below 20,000 IU/ml for most patients. In all patients with HBV-related HCC, no matter in the ILICI group or the Non-ILICI group, a significant reduction in HBV DNA levels after treatment was observed compared to that before treatment. Aside from the inherent effects of antiviral therapy, is it possible that ICIs, as PD-1/PD-L1 pathway inhibitors, may also exhibit antiviral effects against HBV? Previous studies have reported that some ICIs can suppress viral replication in patients with chronic HBV/HCV. However, there are also reports indicating that ICIs treatment in HBV-positive patients may lead to viral reactivation, and other studies have suggested that HBV infection does not affect the clinical outcomes of PD-1 therapy [44–49]. Therefore, the impact of ICIs on viral hepatitis, especially in terms of virus clearance, is still vague and needs more investigation.

Currently, due to patient-specific factors and the irAEs following ICIs therapy, only a small proportion of patients benefit from ICIs treatment. No reliable biomarkers have been definitively identified to predict the ILICI occurrence thus far. Shaheen Khan et al. assessed 40 cytokines in the plasma of patients with various cancers and found that cytokines such as CXCL8-11, CXCL13, IL-6, CXCL2, CCL20 and CCL23 could serve as potential biomarkers

for predicting the occurrence of irAEs [31]. Jia et al. reviewed biomarkers associated with irAEs across different ICIs include C-reactive protein (CRP), cell counts, IL-6, and sCTLA-4 [50]. However, the numbers of cases in these studies are limited or most findings originate from patients with lung cancer or these researches focus primarily on organs like the gastrointestinal tract, endocrine system, skin and lungs, liver-specific markers were not discussed. Although no effective specific biomarkers have been identified so far, researchers have attempted to identify risk factors for ILICI based on clinical factors. Studies suggest that pre-existing conditions like non-alcoholic fatty liver disease, female, history of ICI treatment, the type of ICIs, baseline liver dysfunction and combination therapy may be potential risk factors for ILICI [32, 33, 51]. Likewise, many of the studies referenced are also related to non-liver cancers and it remains uncertain whether these findings apply to ILICI in HCC patients. Our multivariate analysis revealed that diabetes, underlying liver cirrhosis and multiple tumor nodules could be potential risk factors for the occurrence of ILICI. The occurrence of ILICI may relate to the chronic immune imbalance and metabolic disturbances inherent in diabetic patients [52, 53]. Underlying liver cirrhosis was found to be another significant risk factor. In China, the predominant cause of liver cancer is chronic HBV infection and most of them undergoing the “hepatitis-cirrhosis-liver cancer” progression [19–21]. Upon initiation of immunotherapy, the immune system is activated, and immune cells and inflammatory factors attack both tumor cells and normal hepatocytes. For patients with existing cirrhosis, the immunotherapy can lead to liver dysfunction. Another independent risk factor identified is the presence of multiple nodules in HCC (≥ 3 nodules). Multifocal liver cancer often presents in various locations within the liver, suggesting broader areas of involvement and poorer liver reserve. Consequently, when an immune response occurs, more pronounced liver dysfunction may manifest.

Based on the results of the multivariate logistic regression analysis, we established a clinical prediction model aimed at forecasting the occurrence of ILICI. Through ROC analysis, we found that the AUC was 0.701, with sensitivity 0.712, specificity 0.632, PPV 0.720 and NPV 0.630. Although this prediction model demonstrates a certain degree of predictive efficacy, it is not entirely satisfactory. This may be attributed to the retrospective nature of the study, which made it challenging to control for numerous confounding factors. Consequently, a significant number of cases were excluded due to incomplete data, resulting in a relatively small sample size. Furthermore, as discussed above, previous studies have found that factors such as some chemokines, CRP, cell counts, IL-6 possible predict the irAEs occurrence. Thus,

another limitation is that these biomarkers in blood are not included in our study. Patients included in this study were from various departments in our institution, which lead to substantial missing data for potentially relevant biomarkers. This limitation also affects the efficacy of our predictive model. Furthermore, the noninvasive indexes to identify cirrhosis in this study were APRI and FIB-4, which can not completely reflect the situation of cirrhosis. Elastography results may be better than APRI and FIB-4. However, most patients in this study did not perform elastography test. In addition, most HCC patients were HBV-related in this study. Whether the predict model can be applied to other etiologies of HCC such as HCV, alcoholic liver disease or Primary Biliary Cholangitis remains unclear. Thus, we will primarily focus on the aforementioned aspects to develop more robust models for predicting the occurrence of ILICI.

Conclusions

We elucidate the clinical characteristics and independent risk factors of ILICI in HCC patients receiving ICIs therapy, and establish a mathematical model to predict the occurrence risk of ILICI. These findings can assist clinical decision-makers in optimizing ICIs treatment to reduce the incidence of adverse events, arranging monitoring plan and avoiding high risk patients.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12885-025-14540-y>.

Supplementary Material 1.

Acknowledgements

We gratefully acknowledge the invaluable assistance provided by Chunhong Liao in efficiently collecting a substantial number of samples.

Authors' contributions

XYL, HS and LBC designed the work; XYL, HS and JLW assisted in patient enrollment and specimen collection; XYL and HS collected data, analyzed data and drafted the manuscript; LBC edited the draft; All authors reviewed the manuscript.

Funding

This study was supported by the Science and Technology Planning Project Fundamental and Applied Fundamental Research Topics of Guangzhou (2023A04J1091).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author or the first author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of The Third Affiliated Hospital of Sun Yat-sen University. All the procedures performed in the studies were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and the ethical requirements of institutional research ethics committees.

Competing interests

The authors declare no competing interests.

Received: 19 January 2025 / Accepted: 18 June 2025

Published online: 01 July 2025

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