RESEARCH

Nomograms predicting benefit after immunotherapy in oral bifidobacteria supplementation ICC patients: a retrospective study

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Abstract

Purpose The objective of this study was to develop nomograms for predicting outcomes following immunotherapy in patients diagnosed with intrahepatic cholangiocarcinoma (ICC).

Patients and methods A retrospective analysis was conducted on data from 75 ICC patients who received immunotherapy at Jinling Hospital and Drum Hospital. The discriminative power, accuracy, and clinical applicability of the nomograms were assessed using the concordance index (C-index), calibration curve, and decision curve analysis (DCA). The predictive performance of the nomograms for overall survival (OS) and progression-free survival (PFS) was evaluated using the area under the receiver operating characteristic (ROC) curve. Kaplan-Meier curves were also generated for validation purposes.

Results Multivariable analysis identified independent prognostic factors for OS, including CA19-9 levels, portal vein tumor thrombus (PVTT) grade, bifidobacteria administration, and surgery. The C-index of the nomogram for OS prediction was 0.722 (95% confidence interval [CI]: 0.661–0.783). Independent prognostic factors for PFS included CA19-9 levels, albumin, and bilirubin, with a C-index of 0.678 (95% CI: 0.612–0.743) for the nomogram predicting PFS. Calibration curves demonstrated strong concordance between predicted and observed outcomes, while DCA and Kaplan-Meier curves further supported the clinical utility of the nomogram.

Conclusion The nomogram developed in this study demonstrated favorable performance in predicting the prognosis of ICC patients undergoing immunotherapy. Additionally, our findings, for the first time, identified

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probiotics as a potential prognostic marker for immunotherapy. This prognostic model has the potential to enhance patient selection for immunotherapy and improve clinical decision-making.

Keywords Immunotherapy, Nomogram, Prognostic factor, Intrahepatic cholangiocarcinoma

Introduction

Intrahepatic cholangiocarcinoma (ICC) is situated proximal to the secondary bile ducts, within the hepatic parenchyma. It constitutes approximately 10% of all primary liver malignancies, ranking as the second most prevalent primary hepatic neoplasm following hepatocellular carcinoma (HCC) [1]. The incidence and mortality rates of ICC have experienced a notable escalation over the past few decades [2, 3]. Surgical excision remains the cornerstone of therapeutic intervention [4]. However, only a minority of potentially operable patients undergo surgical resection [5]. The recurrence rate post-surgical resection remains substantial, ranging from 50 to 60%, with a median disease-free survival of under 26 months [6]. For those patients subjected to adjuvant chemotherapy subsequent to surgery, the median survival extends to approximately 51.1 months [7]. Unfortunately, the prognosis for advanced ICC cases is even grimmer, with a median overall survival (OS) of less than 1 year following the standard first-line chemotherapy regimen [4].

Immunotherapy, particularly with immune checkpoint inhibitors (ICIs), has rapidy advanced in recent years [8]. Heterogeneities in the etiology, tumor immune microenvironment, and genetic makeup of intrahepatic cholangiocarcinoma (ICC) lead to varying responses to ICIs [9–11]. Monotherapy achieves objective response rates (ORRs) within the range from 3–22% [12]. Notably, combining immunotherapy with chemotherapy, targeted therapy, or conventional treatments significantly improves efficacy [13–15]. Recent studies have also found that gut bacteria are associated with immunotherapy outcomes, with bifidobacterium showing strong effects [16, 17], significantly boosting the effectiveness of immunotherapy. Despite the generally favorable safety profile of ICIs, a minority of patients may encounter severe immune-related adverse effects [18], a risk that escalates notably when combined with other agents [19, 20]. Currently, there is limited clinical data available for ICC, and definitive prognostic markers remain elusive.

Prognostic nomograms have been systematically devised for various cancer types. In this study, we leveraged baseline clinical parameters within a cohort of 75 ICC patients receiving treatment regimens other than immunotherapy, including with or without the the oral administration of bifidobacteria. These nomograms aim to accurately predict patient outcomes, providing a robust framework for clinical prognosis in ICC.

Patients and methods

Patients

We conducted a retrospective study, developing nomograms to predict prognosis after immunotherapy using data from the Jinling Hospital and Drum Hospital.

We screened patients who underwent immunotherapy between September 2019 and November 2023. Eligible participants received ICIs through intravenous injection at intervals of three to four weeks. The specific ICIs included sintilimab, camrelizumab, tislelizumab, pembrolizumab (each at 200 mg), and toripalimab (240 mg). Inclusion criteria required patients to have available baseline data and documented survival outcomes (refer to Table 1). Patients with histopathologically confirmed mixed types of liver cancer were excluded from the study. Ethical approval for all research protocols was obtained from the Ethics Committee of Jinling Hospital, adhering to the principles outlined in the Declaration of Helsinki.

Clinical data

We gathered 38 pretherapeutic parameters for analysis, as outlined in Table 1. These parameters encompassed demographic details such as age and gender, baseline laboratory assessments and imaging results, historical treatment records, details of treatment regimens, presence of portal vein tumor thrombosis (PVTT), use of probiotics, hepatitis B virus (HBV) status, identification of metastatic and progression sites, presence of liver cirrhosis, presence of ascites, engagement in treatment beyond progression (TBP), the number of treatment lines, Eastern Cooperative Oncology Group performance status (ECOG PS), and the occurrence of adverse events (AE).

Outcomes

The principal endpoints for the nomograms in this study are OS and PFS. OS for all patients, including those who underwent surgery, is defined as the time from the start of immunotherapy to death from any cause, while PFS is delineated as the interval from the initiation of immunotherapy to the onset of disease progression or death from any cause. ICC progression is ideally comfirmed through subsequent assessments, with a minimum interval of 4 weeks, adhering to the immunotherapy response evaluation criteria in solid

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tumors (iRECIST) version 1.1. These criteria include the following categories: complete response (iCR), partial response (iPR), stable disease (iSD), and progressive disease (iPD).

Statistical analysis

The sample sizes were determined based on available data, and all statistical analyses were conducted using SPSS software version 22.0 and R programming. Descriptive statistics are presented as frequencies and proportions for categorical variables, and as medians or means for continuous variables. OS and PFS with 95% confidence intervals (CI) were estimated employing the Kaplan-Meier method. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. Univariate and multivariate Cox regression analyses were conducted to identify independent statistically significant prognostic factors, with results expressed as hazard ratios (HR) and corresponding 95% CI. A significance level of $p < 0.05$ was adopted for statistical interpretation.

Result

Patient characteristics

Between September 2019 and November 2023, a total of 92 patients underwent screening, of whom 75 (81.5%) met the eligibility criteria and were stratified into two cohorts based on their outcomes—deaths ($n = 56$) and survivors ($n = 19$) (refer to Fig. 1). Among the eligible participants, 53 (70.07%) were male and 22 (29.03%) female. Prior medical interventions included 35 (46.67%) patients who had undergone surgery, 18 (24%) who had received radiotherapy, 21 (28%) who had received targeted therapy, 49 (65.33%) who received chemotherapy, 14 (18.67%) who underwent transarterial chemoembolization (TACE), and 8 (10.67%) who were on long-term oral bifidobacterial administration. Additionally, 38 (50.67%) patients had a history of HBV infection. Other pertinent medical backgrounds included 23 (30.67%) individuals with liver cirrhosis and 30 (40%) presenting with ascites. Performance status, as measured by Eastern Cooperative Oncology Group (ECOG) Performance Status, indicated that 62 (82.67%) patients had scores of 0–1. Furthermore, 58 (77.33%) patients fell within Child-Pugh class A. Treatment-related adverse events (AEs) of Grade 3/4 severity were reported in 28 (37.33%) cases. Baseline characteristics and indicators were evenly distribution between the death and survival cohorts, except for the variable of surgical intervention, as detailed in Table 1.

Independent prognostic factors for OS and PFS

We subsequently conducted an analysis to assess the association between each variable and OS, as presented in Table 2. Univariate analysis identified significant associations with CA19-9 levels, albumin concentrations, metastatic sites, PVTT grade, probiotic usage, surgical interventions, prior radiotherapy, presence of ascites, treatment-related AEs, and Child-Pugh stage, indicating their potential influence as OS predictors. A subsequent multivariate analysis revealed that CA19-9 levels, PVTT grade, administration of bifidobacteria, and prior surgery emerged as independent risk factors impacting patients with cholangiocellular carcinoma undergoing immunotherapy.

The association between variables and PFS underwent a similar analysis. As depicted in Table 3, univariate analysis revealed significant associations with CA19-9 levels, albumin concentrations, bilirubin levels, prior surgical interventions, ECOG PS, and Child-Pugh stage, marking them as potential determinants. Subsequent multivariate analysis identified CA19-9 levels, albumin concentrations, and bilirubin levels as independent risk factors impacting PFS.

Prognostic Nomogram for OS and PFS

The prognostic nomogram, encompassing all pertinent independent factors significantly associated with OS subsequent to immunotherapy for ICC, is sho in Fig. 2a. This nomogram incorporates variables such as CA19-9 levels, PVTT grade, bifidobacteria administration, and surgical interventions. The calibration plot, showing the probability of survival at 6, 12, or 24 months, demonstrated strong concordance between the nomogram-based predictions and observed outcomes, as depicted in Fig. 2b. The Concordance Index (C-index) for OS prediction was calculated to be 0.722 (95% CI, 0.661 to 0.783).

Applying the same methodological approach, nomograms were developed to predict PFS in ICC patients at 6, 12, and 24 months, as shown in Fig. 2c. The figures highlight CA19-9 levels, albumin concentrations, and bilirubin levels as pivotal factors collaboratively influencing the prediction of PFS in cholangiocarcinoma patients subjected to immunotherapy. Consistent with the OS nomogram, the calibration plots show a good alignment between nomogram-derived predictions and actual observed outcomes, as depicted in Fig. 2d. The C-index for PFS prediction was determined to be 0.678 (95% CI, 0.612–0.743).

Validation of predictive accuracy of the Nomogram for OS and PFS

To enhance the discriminative capacity of the nomogram predictions, Receiver Operating Characteristic

Table 1 Patient baseline characteristics

	Total (*n* = 75)	Alive(*n* = 19)	Dead(*n* = 56)	*p*
month, Median (Q1,Q3)	10.2 (4.45, 18.55)	24.1 (3.85, 33.45)	9.8 (4.65, 14.85)	0.111
Age				0.592
<40	7 (9.33)	3 (15.79)	4 (7.14)	
40–70	61 (81.33)	15 (78.95)	46 (82.14)	
>70	7 (9.33)	1 (5.26)	6 (10.71)	
AFP				0.434
≤10ng/ml	66 (88)	18 (94.74)	48 (85.71)	
>10ng/ml	9 (12)	1 (5.26)	8 (14.29)	
CA19-9				0.747
≤37U/ml	59 (78.67)	16 (84.21)	43 (76.79)	
>37U/ml	16 (21.33)	3 (15.79)	13 (23.21)	
NLR				0.321
<2	5 (6.67)	0 (0)	5 (8.93)	
≥2	70 (93.33)	19 (100)	51 (91.07)	
PLR				0.747
<100	15 (20)	3 (15.79)	12 (21.43)	
≥100	60 (80)	16 (84.21)	44 (78.57)	
Hemoglobin				0.84
<120 g/L	39 (52)	9 (47.37)	30 (53.57)	
≥120 g/L	36 (48)	10 (52.63)	26 (46.43)	
White blood cells				0.64
<3.5×10^9/L	6 (8)	2 (10.53)	4 (7.14)	
≥3.5×10^9/L	69 (92)	17 (89.47)	52 (92.86)	
Bilirubin				0.999
≤20.5 mol/L	65 (86.67)	17 (89.47)	48 (85.71)	
>20.5 mol/L	10 (13.33)	2 (10.53)	8 (14.29)	
Albumin				0.496
<35 g/L	14 (18.67)	2 (10.53)	12 (21.43)	
≥35 g/L	61 (81.33)	17 (89.47)	44 (78.57)	
LDH				0.286
<120U/L	2 (2.67)	0 (0)	2 (3.57)	
120U/L-246U/L	52 (69.33)	16 (84.21)	36 (64.29)	
>246U/L	21 (28)	3 (15.79)	18 (32.14)	
Types of ICIs Drugs				0.45
Camrelizumab	36 (48)	7 (36.84)	29 (51.79)	
Sintilimab	24 (32)	8 (42.11)	16 (28.57)	
Others	15 (20)	4 (21.05)	11 (19.64)	
Liver metastas				0.144
No	20 (26.67)	8 (42.11)	12 (21.43)	
Yes	55 (73.33)	11 (57.89)	44 (78.57)	
Lung metastasis				0.196
No	48 (64)	15 (78.95)	33 (58.93)	
Yes	27 (36)	4 (21.05)	23 (41.07)	
Lymph node metastasis				0.628
No	21 (28)	4 (21.05)	17 (30.36)	
Yes	54 (72)	15 (78.95)	39 (69.64)	
Bone metastasis				0.747
No	59 (78.67)	16 (84.21)	43 (76.79)	
Yes	16 (21.33)	3 (15.79)	13 (23.21)	
Other metastasis				0.33
No	59 (78.67)	17 (89.47)	42 (75)	
Yes	16 (21.33)	2 (10.53)	14 (25)	
PVTT grade				0.133

Table 1 (continued)

	Total (*n* = 75)	Alive(*n* = 19)	Dead(*n* = 56)	*p*
<3	57 (76)	17 (89.47)	40 (71.43)	
≥3	18 (24)	2 (10.53)	16 (28.57)	
Probiotics				0.19
No	67 (89.33)	15 (78.95)	52 (92.86)	
Yes	8 (10.67)	4 (21.05)	4 (7.14)	
Gender				0.999
Male	54 (72)	14 (73.68)	40 (71.43)	
Female	21 (28)	5 (26.32)	16 (28.57)	
Surgery				**0.003**
No	40 (53.33)	4 (21.05)	36 (64.29)	
Yes	35 (46.67)	15 (78.95)	20 (35.71)	
Radiotherapy				0.212
No	57 (76)	12 (63.16)	45 (80.36)	
Yes	18 (24)	7 (36.84)	11 (19.64)	
TACE				0.326
No	61 (81.33)	14 (73.68)	47 (83.93)	
Yes	14 (18.67)	5 (26.32)	9 (16.07)	
HBV				0.320
No	37 (49.33)	7 (36.84)	30 (53.57)	
Yes	38 (50.67)	12 (63.16)	26 (46.43)	
Liver Cirrhosis				0.999
No	52 (69.33)	13 (68.42)	39 (69.64)	
Yes	23 (30.67)	6 (31.58)	17 (30.36)	
Ascites				0.255
No	45 (60)	14 (73.68)	31 (55.36)	
Yes	30 (40)	5 (26.32)	25 (44.64)	
Progression liver				0.999
No	14 (18.67)	3 (15.79)	11 (19.64)	
Yes	61 (81.33)	16 (84.21)	45 (80.36)	
Progression lung				0.747
No	59 (78.67)	16 (84.21)	43 (76.79)	
Yes	16 (21.33)	3 (15.79)	13 (23.21)	
Progression lymph node				0.999
No	52 (69.33)	13 (68.42)	39 (69.64)	
Yes	23 (30.67)	6 (31.58)	17 (30.36)	
Progression bone				0.999
No	68 (90.67)	17 (89.47)	51 (91.07)	
Yes	7 (9.33)	2 (10.53)	5 (8.93)	
Progression others				0.999
No	68 (90.67)	17 (89.47)	51 (91.07)	
Yes	7 (9.33)	2 (10.53)	5 (8.93)	
TBP				0.745
No	47 (62.67)	13 (68.42)	34 (60.71)	
Yes	28 (37.33)	6 (31.58)	22 (39.29)	
Targeted Therapy				0.06
No	54 (72)	10 (52.63)	44 (78.57)	
Yes	21 (28)	9 (47.37)	12 (21.43)	
Chemotherapy				0.104
No	26 (34.67)	10 (52.63)	16 (28.57)	
Yes	49 (65.33)	9 (47.37)	40 (71.43)	
Drug Administration Sequence				0.578
TKI	36 (48)	7 (36.84)	29 (51.79)	
ICIs	10 (13.33)	3 (15.79)	7 (12.5)	

Table 1 (continued)

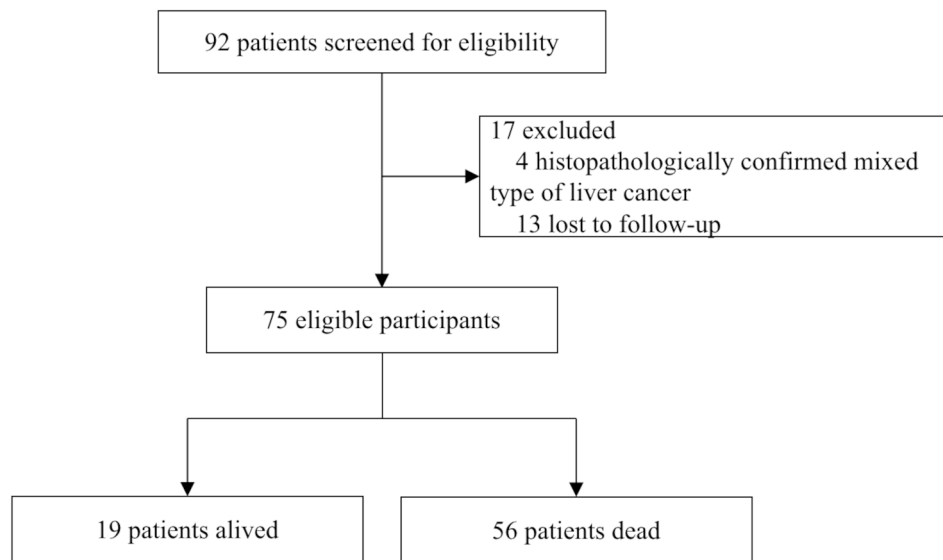|	Total (*n* = 75)	Alive(*n* = 19)	Dead(*n* = 56)	*p*
Synchronous	29 (38.67)	9 (47.37)	20 (35.71)	
Line				0.392
1	46 (61.33)	11 (57.89)	35 (62.5)	
2	16 (21.33)	6 (31.58)	10 (17.86)	
>2	13 (17.33)	2 (10.53)	11 (19.64)	
ECOG				0.727
1	62 (82.67)	15 (78.95)	47 (83.93)	
≥2	13 (17.33)	4 (21.05)	9 (16.07)	
AE				0.745
≤Grade 2	47 (62.67)	13 (68.42)	34 (60.71)	
Grade3/4	28 (37.33)	6 (31.58)	22 (39.29)	
Child Pugh stage				0.496
A	61 (81.33)	17 (89.47)	44 (78.57)	
B	14 (18.67)	2 (10.53)	12 (21.43)	

Fig. 1 Study selection process

(ROC) curves were calculated for OS and PFS. As depicted in Fig. 3a, the Area Under the Curve (AUC) values for the probability of 6-Month survival, 12-Month survival, and 24-Month survival were determined as 0.749 (0.628–0.871), 0.826 (0.730–0.922), and 0.849 (0.730–0.922), respectively. Decision Curve Analysis (DCA) curve further supports the model's clinical utility within this prognostic range, as illustrated in Fig. 3b-d. For PFS, the AUC values for the 6, 12, and 24-Month time points were calculated as 0.743 (0.647–0.839), 0.760 (0.687–0.834), and 0.715 (0.687–0.834), respectively (Fig. 3e).

Utilizing the nomogram scores for OS and their corresponding AUC values, we computed risk scores and determined optimal cut-off values for stratifying patients. Patients were divided into a high-risk group (>141.7) and a low-risk group (≤141.7). The high-risk group had significantly poorer OS [7.6 months (95% CI 0.375–0.689) vs. 23.2 months (95% CI 0.363–0.749), $p < 0.0001$] as shown in Fig. 3f. Similarly, stratification based on the nomogram score for PFS [low-risk (≤1.888), high-risk (>1.888)] revealed a statistically significant difference between the two groups [3.1 months (95% CI 0.334–0.730) vs. 8.1 months (95% CI 0.380–0.701), $p < 0.0001$], as depicted in Fig. 3g.

Discussion

In this study, a comprehensive analysis was conducted on the clinical data of 75 patients diagnosed with ICC who underwent immunotherapy. COX regression analysis was employed to identify independent prognostic factors, including CA19-9, albumin levels, presence of metastases at other sites, PVTT grade, administration of bifidobacteria, surgical intervention, radiotherapy,

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Table 2 Uni- and multivariate analyses for OS

	Univariate			Multivariate		
	HR	95%CI	*p*	HR	95%CI	*p*
Age, years						
<40	Ref					
40–70	1.007	0.360–2.815	0.990			
>70	0.603	0.168–2.167	0.438			
AFP						
≤10ng/ml	Ref					
>10ng/ml	1.841	0.864–3.922	0.114			
CA19-9						
≤37U/ml	Ref			Ref		
>37U/ml	2.819	1.443–5.509	**0.002**	2.117	1.039–4.316	**0.039**
NLR						
<2	Ref					
≥2	1.002	0.398–2.524	0.997			
PLR						
<100	Ref					
≥100	1.032	0.544–1.958	0.922			
Hemoglobin						
<120 g/L	Ref					
≥120 g/L	0.834	0.492–1.414	0.501			
White blood cells						
<3.5×10^9/L	Ref					
≥3.5×10^9/L	1.578	0.569–4.376	0.381			
Bilirubin						
≤20.5 mol/L	Ref					
>20.5 mol/L	1.490	0.700-3.174	0.301			
Albumin						
<35 g/L	Ref			Ref		
≥35 g/L	0.257	0.131–0.501	**<0.001**	0.497	0.234–1.055	0.069
LDH						
<120U/L	Ref					
120U/L-246U/L	0.438	0.104–1.841	0.260			
>246U/L	0.505	0.115–2.215	0.365			
Types of ICIs Drugs						
Camrelizumab	Ref					
Sintilimab	0.765	0.413–1.418	0.395			
Others	0.863	0.424–1.753	0.683			
Liver metastas						
No	Ref					
Yes	1.900	0.996–3.622	0.051			
Lung metastasis						
No	Ref					
Yes	1.453	0.852–2.479	0.171			
Lymph node metastasis						
No	Ref					
Yes	0.782	0.439–1.394	0.405			
Bone metastasis						
No	Ref					
Yes	1.232	0.661–2.297	0.512			
Other metastasis						
No	Ref					
Yes	1.701	0.921–3.141	0.090			
PVTT grade						

Table 2 (continued)

	Univariate			Multivariate		
	HR	95%CI	*p*	HR	95%CI	*p*
<3	Ref			Ref		
≥3	2.580	1.418–4.695	**0.002**	2.437	1.181–5.028	**0.016**
Probiotics						
No	Ref			Ref		
Yes	0.339	0.122–0.942	**0.038**	0.29	0.097–0.867	**0.027**
Gender						
Male	Ref					
Female	1.036	0.578–1.857	0.905			
Surgery						
No	Ref			Ref		
Yes	0.384	0.218–0.676	**0.001**	0.484	0.267–0.878	**0.017**
Radiotherapy						
No	Ref			Ref		
Yes	0.492	0.254–0.954	**0.036**	0.557	0.284–1.094	0.089
TACE						
No	Ref					
Yes	0.804	0.392–1.645	0.550			
HBV						
No	Ref					
Yes	0.688	0.405–1.170	0.168			
Liver Cirrhosis						
No	Ref					
Yes	1.017	0.572–1.809	0.953			
Ascites						
No	Ref					
Yes	1.711	1-2.926	0.050			
Progression liver						
No	Ref					
Yes	1.013	0.521–1.969	0.971			
Progression lung						
No	Ref					
Yes	1.022	0.547–1.907	0.946			
Progression lymph node						
No	Ref					
Yes	0.921	0.521–1.63	0.778			
Progression bone						
No	Ref					
Yes	1.280	0.509–3.217	0.600			
Progression others						
No	Ref					
Yes	0.972	0.385–2.454	0.952			
TBP						
No	Ref					
Yes	0.764	0.446–1.309	0.327			
Targeted Therapy						
No	Ref					
Yes	0.647	0.339–1.235	0.187			
Chemotherapy						
No	Ref					
Yes	1.344	0.751–2.405	0.319			
Drug Administration Sequence						
TKI	Ref					

Table 2 (continued)

	Univariate			Multivariate		
	HR	95%CI	*p*	HR	95%CI	*p*
ICIs	0.704	0.305–1.628	0.412			
Synchronous	0.835	0.466–1.495	0.544			
Line						
1	Ref					
2	1.122	0.552–2.28	0.750			
>2	1.248	0.627–2.483	0.529			
ECOG						
1	Ref					
≥2	1.199	0.586–2.456	0.619			
AE						
≤Grade 2	Ref					
Grade3/4	1.284	0.746–2.209	0.367			
Child Pugh stage						
A	Ref			Ref		
B	2.120	1.107–4.06	**0.023**	1.083	0.528–2.219	0.828

combined peritoneal effusion, treatment-related adverse events, and Child-Pugh stage. These factors were incorporated into nomogram models, providing valuable insights for clinical diagnosis and treatment decision-making.

Several ICIs have shown potent and durable antitumor activity and have been approved by the FDA for treating of various malignancies [21–23]. Immunotherapy has demonstrated enhanced efficacy in viral infection-associated tumors, including head and neck cancer, Hodgkin lymphoma, and HCC, due to neoantigens linked to viral infections [24–26]. However, despite the association of ICC with chronic infections like hepatic schistosomiasis, viral hepatitis B and C, and bacterial septic cholangitis [27], no significant benefit of immunotherapy monotherapy in ICC has been demonstrated. Recent studies increasingly show that combining immunotherapy with other treatment regimens can lead to prolonged survival and substantial clinical benefits for patients [14, 28–31].

While immunotherapy is generally considered safe, a subset of patients may encounter severe immune-related adverse events, especially when combined with other therapies [32]. As a result, there is a strong need for reliable immunotherapy biomarkers that can effectively identify individuals who would benefit from such treatment. Recognized biomarkers currently include tumor mutational burden (TMB) [33], microsatellite instability-high (MSI-H) status [34], and programmed cell death ligand 1 (PD-L1) expression. However, these markers have limitations and do not fully provide patient prognosis [21]. With the increasing use of immunotherapy, there is an urgent demand for more precise biomarkers that can reliably forecast patient outcomes.

Nomograms are widely used in tumor prognostic models and have shown to enhance predictive accuracy [35–37]. In this study, we developed and validated a prognostic nomogram for ICC by incorporating independent risk factors identified through Cox regression analysis. This nomogram allowed us to effectively identify, during follow-up, patients who would benefit from treatment. Additionally, two separate line graphs were developed to predict OS and PFS in patients undergoing immunotherapy, and these predictions were subsequently validated. Our findings confirmed that pre-treatment CA19-9 levels, cancer embolism grade, bifidobacteria administration, and surgical intervention were significant factors influencing OS prediction for cholangiocarcinoma patients receiving immunotherapy. Furthermore, CA19-9 levels, albumin levels, and bilirubin levels were key factors that predicting PFS.

The role of microbes in the development, diagnosis, and treatment remains a topic of ongoing debate and research. Alterations in the gut microbiota modulate the immune response [38]. Bacterial outer membrane vesicles (OMVs) can inhibit tumor growth and metastasis through interferon-gamma-mediated antitumor responses [39]. Research by Noriho Iida et al. indicated that cancer-bearing mice treated with antibiotics or kept under sterile conditions exhibited poor responses to therapeutic interventions, suggesting that a symbiotic microbiota is essential for effective anti-tumor therapy by modulating the tumor microenvironment [39, 40]. Similarly, Lijuan Wang et al. founded that certain gut microflora can induce TNF-α production while down-regulating PD-L1 expression [41]. Lukas F Mager et al. demonstrated that specific bacteria, including *Bifidobacterium pseudolongum*,

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Table 3 Uni- and multivariate analyses for PFS

	Univariate			Multivariate		
	HR	95%CI	*p*	HR	95%CI	*p*
Age						
< 40	Ref					
40–70	1.087	0.388–3.044	0.874			
> 70	0.823	0.219–3.094	0.773			
AFP						
≤ 10ng/ml	Ref					
> 10ng/ml	1.162	0.495–2.727	0.731			
CA19-9						
≤ 37U/ml	Ref			Ref		
> 37U/ml	2.112	1.075–4.15	**0.030**	2.495	1.196–5.205	**0.015**
NLR						
< 2	Ref					
≥ 2	1.429	0.443–4.603	0.550			
PLR						
< 100	Ref					
≥ 100	1.136	0.551–2.34	0.730			
Hemoglobin						
< 120 g/L	Ref					
≥ 120 g/L	1.142	0.662–1.97	0.634			
White blood cells						
< 3.5 × 10^9/L	Ref					
≥ 3.5 × 10^9/L	1.020	0.402–2.584	0.967			
Bilirubin						
≤ 20.5 mol/L	Ref			Ref		
> 20.5 mol/L	3.720	1.666–8.308	**0.001**	2.858	1.125–7.259	**0.027**
Albumin						
< 35 g/L	Ref			Ref		
≥ 35 g/L	0.209	0.093–0.47	**< 0.001**	0.227	0.095–0.544	**0.001**
LDH						
< 120U/L	Ref					
120U/L-246U/L	0.576	0.137–2.414	0.450			
> 246U/L	0.429	0.095–1.926	0.269			
Types of ICIs Drugs						
Camrelizumab	Ref					
Sintilimab	0.711	0.38–1.331	0.286			
Others	0.830	0.4-1.726	0.619			
Liver metastas						
No	Ref					
Yes	1.523	0.81–2.863	0.192			
Lung metastasis						
No	Ref					
Yes	1.217	0.683–2.169	0.506			
Lymph node metastasis						
No	Ref					
Yes	0.729	0.393–1.354	0.317			
Bone metastasis						
No	Ref					
Yes	1.496	0.767–2.916	0.237			
Other metastasis						
No	Ref					
Yes	1.437	0.747–2.766	0.277			
PVTT grade						

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Table 3 (continued)

	Univariate			Multivariate		
	HR	95%CI	*p*	HR	95%CI	*p*
<3	Ref					
≥3	1.813	0.958–3.431	0.067			
Bifidobacteria						
No	Ref					
Yes	0.424	0.166–1.082	0.073			
Gender						
Male	Ref					
Female	0.960	0.524–1.759	0.894			
Surgery						
No	Ref			Ref		
Yes	0.557	0.313–0.994	**0.048**	0.719	0.393–1.314	0.283
Radiotherapy						
No	Ref					
Yes	0.835	0.459–1.52	0.555			
TACE						
No	Ref					
Yes	0.960	0.467–1.975	0.912			
HBV						
No	Ref					
Yes	1.176	0.679–2.036	0.562			
Liver Cirrhosis						
No	Ref					
Yes	1.107	0.597–2.053	0.746			
Ascites						
No	Ref					
Yes	1.272	0.717–2.256	0.410			
Progression liver						
No	Ref					
Yes	0.773	0.393–1.521	0.456			
Progression lung						
No	Ref					
Yes	0.770	0.385–1.541	0.460			
Progression lymph node						
No	Ref					
Yes	0.998	0.562–1.774	0.995			
Progression bone						
No	Ref					
Yes	2.152	0.897–5.163	0.086			
Progression others						
No	Ref					
Yes	1.169	0.495–2.761	0.722			
TBP						
No	Ref					
Yes	1.122	0.648–1.942	0.682			
Targeted Therapy						
No	Ref					
Yes	0.906	0.487–1.685	0.755			
Chemotherapy						
No	Ref					
Yes	0.975	0.544–1.75	0.933			
Drug Administration Sequence						
TKI	Ref					

Table 3 (continued)

	Univariate			Multivariate		
	HR	95%CI	*p*	HR	95%CI	*p*
ICIs	0.706	0.306–1.627	0.413			
Synchronous	0.637	0.348–1.167	0.144			
Line						
1	Ref					
2	1.316	0.619–2.796	0.475			
>2	1.555	0.796–3.038	0.196			
ECOG						
1	Ref			Ref		
≥2	2.112	1.034–4.317	**0.040**	1.666	0.765–3.628	0.198
AE						
≥Grade 2	Ref					
Grade3/4	1.057	0.599–1.865	0.848			
Child Pugh stage						
A	Ref			Ref		
B	2.482	1.207–5.104	**0.013**	1.237	0.539–2.842	0.616
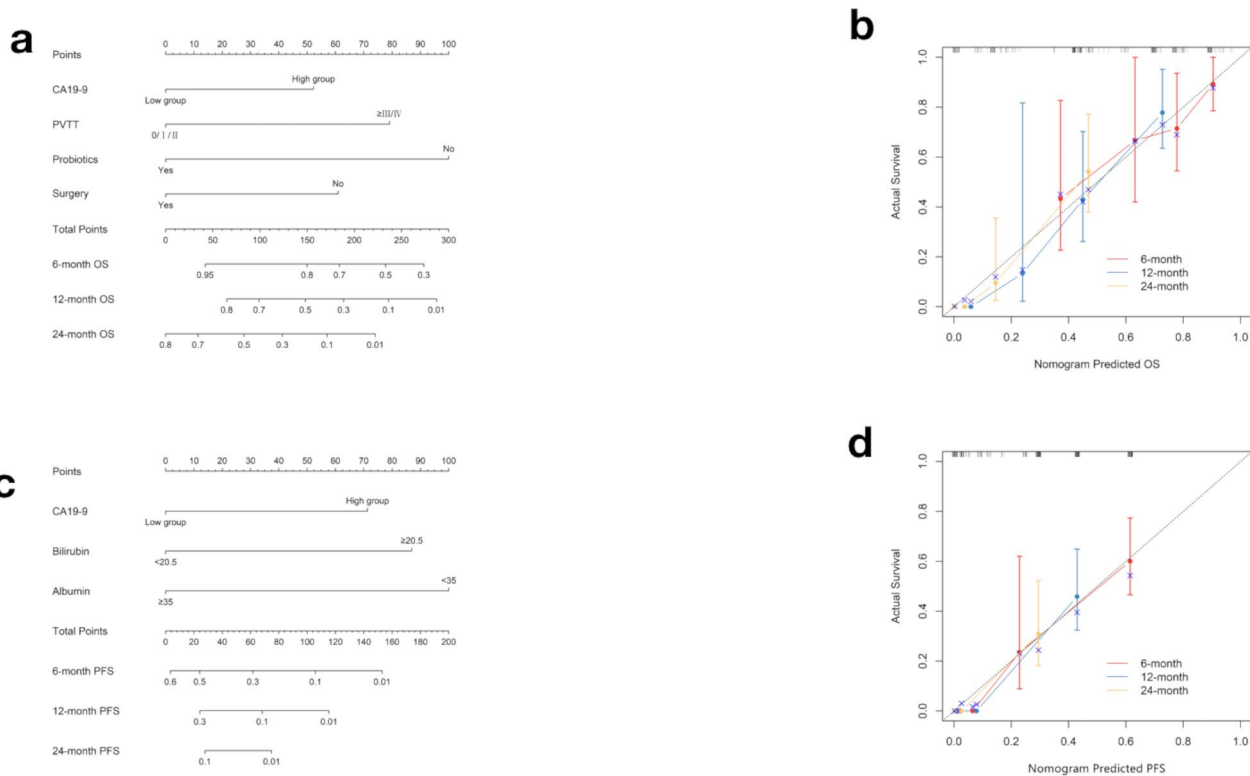
Fig. 2 (**a**) Nomogram for predicting probability of OS at 6, 12 and 24-Months. (**b**) Calibration plots of OS probabilities at 6, 12, 24-Month. (**c**) Nomogram for predicting probability of PFS at 6, 12 and 24-Months. (**d**) Calibration plots of PFS probabilities at 6, 12, 24-Month

Lactobacillus johnsonii, and *Olsenella species*, significantly enhanced the efficacy of ICIs, further supporting that microorganisms can enhance immunotherapy [42]. In our study, some patients had been taking oral bifidobacteria long-term to regulate their gut flora. The results confirmed that these patients showed a significant OS advantage. However, the specific mechanisms behind this phenomenon remain unexplored. We plan to continue collecting relevant data and conducting mechanistic studies to clarify these mechanisms.

In conclusion, the nomogram developed in this study demonstrates a high level of accuracy in predicting the prognosis of immunotherapy in ICC patients.
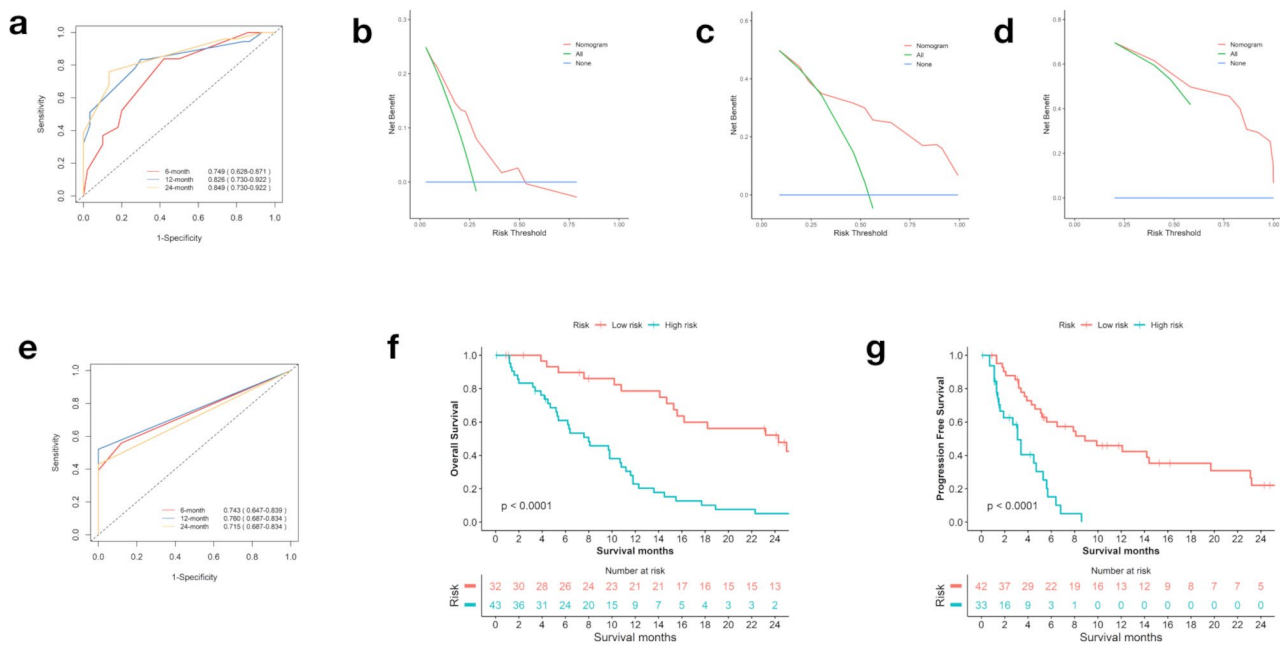
Fig. 3 (**a**) ROC curves in 6, 12, and 24-Months for the OS nomogram. (**b-d**) DCA curve for the OS nomogram. (**e**) ROC curves in 6, 12, and 24-Months for the PFS nomogram. (**f**) OS curves by risk groups. (**g**) PFS curves by risk groups

Additionally, our findings provide novel evidence suggesting that oral bifidobacteria supplementation can significantly extend OS in ICC patients receiving immunotherapy. However, the low incidence of ICC and the limited sample size in this study require further validation of the nomogram's predictive capacity. To enhance the robustness and generalizability of our findings, future research will involve a larger patient cohort.

Author contributions
Conception/Design: S.Z., Y.J., J.S.; Provision of study material/patients: S.Z., Y.J., M.Z., B.L., C.C.; Collection and/or assembly of data: S.Z., J.Z., M.Z., X.L., C.C.; Data analysis and interpretation: S.Z., Y.J., J.Z., B.L., J.S., C.C.; Manuscript writing: S.Z., Y.J., C.C.; Final approval of manuscript: X.L., J.S., C.C.; all authors have read and approved the article.

Data availability
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Ethics Statement
Ethical approval for all research protocols was obtained from the Ethics Committee of Jinling Hospital, adhering to the principles outlined in the Declaration of Helsinki. The patients provided written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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