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# Clinical significance of cytokeratin 19 expression in hepatocellular carcinoma

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## Abstract

**Aim:** This study aimed to compare the prognostic outcomes of cytokeratin (CK) 19-positive and CK19-negative hepatocellular carcinoma (HCC), focusing on clinicopathological features and the impact of targeted therapy in CK19-positive patients.

**Methods:** A retrospective analysis was performed on 310 HCC patients who underwent curative resection between 2010 and 2020 at the First Affiliated Hospital of Nanjing Medical University. Among them, 102 were CK19-positive and 168 were CK19-negative. Multivariate Cox regression was used to identify independent predictors of overall survival (OS) and recurrence-free survival (RFS). Kaplan-Meier survival curves were generated from the Cox model.

**Results:** CK19-positive patients exhibited significantly poorer tumor differentiation ( $P < 0.001$ ), increased microvascular invasion ( $P = 0.010$ ), elevated  $\alpha$ -fetoprotein (AFP) ( $P = 0.0001$ ) and were more often asymptomatic at diagnosis ( $P = 0.014$ ). The median survival time (MST) was 22.1 months in CK19-positive versus 60.3 months in CK19-negative patients. Among those with recurrence, MST was 56.4 months for CK19-positive and 101.6 months for CK19-negative patients. CK19 status significantly impacted OS ( $P < 0.001$ ) and RFS ( $P = 0.006$ ) in advanced-stage cases. Independent prognostic factors for RFS included cirrhosis, tumor size, number of tumors,



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macrovascular invasion, poor differentiation, and CK19 expression. Microvascular invasion and Child-Pugh classification were additional predictors of OS. Targeted therapies did not significantly improve outcomes in CK19-positive patients.

**Conclusions:** CK19-positive HCC is associated with more aggressive tumor behavior, higher recurrence, and poorer survival. Targeted therapy provided no significant survival benefit in this study.

**Keywords:** Hepatocellular carcinoma, cytokeratin 19, overall survival, recurrence-free survival, targeted therapy

## INTRODUCTION

From 2018 to 2022, hepatocellular carcinoma (HCC) has consistently been the 6th most prevalent cancer worldwide. During this period, it has risen from the fourth to the third leading cause of cancer-related deaths, accounting for approximately 758,000 deaths in 2022<sup>[1-3]</sup>. Asia bears the highest burden, representing 75% of global HCC cases, with China alone contributing to 50% of the total<sup>[4]</sup>. Despite the treatment techniques currently available, such as surgical resection, ablation, transarterial chemoembolization (TACE), radiotherapy, and systemic therapy, prolonging life expectancy in HCC patients remains a constant challenge. Until now, the 5-year overall survival rate has been 30%-40% after resections, with 70% of recurrences occurring within 5 years<sup>[5]</sup>. The implementation of semi-annual surveillance programs, particularly among patients with cirrhosis, has markedly improved early tumor detection rates. A meta-analysis encompassing 15,158 patients demonstrated that surveillance is associated with a 2.08-fold increase in early-stage HCC detection and a 2.24-fold increase in the receipt of curative treatments, ultimately leading to enhanced overall survival (OR 1.90)<sup>[6]</sup>. Concurrently, the etiology of HCC is evolving, with metabolic dysfunction-associated fatty liver disease (MAFLD) emerging as a predominant risk factor. MAFLD now affects approximately 30% of adults globally, with higher prevalence in Western countries, and is projected to contribute to a 122% increase in MAFLD-related HCC cases by 2030<sup>[7]</sup>. According to SHARP and Asia-Pacific trials, advances in targeted therapy have revealed Sorafenib as the first-line therapy since 2008 until the exploration of Lenvatinib, which revolutionized the landscape in 2018<sup>[8-10]</sup>. Although Lenvatinib, as a multitarget kinase inhibitor, has proven to be effective in the treatment of advanced HCC<sup>[11]</sup>, its therapeutic effect on CK19-positive HCC, among other agents, remains unclear.

Consequently, researchers have attempted to analyze the various subtypes of HCC in order to improve patient prognosis and treatment outcomes. Prior research has classified HCC into two major molecular subtypes: proliferative and non-proliferative<sup>[12-14]</sup>. CKs are polygenic biomarkers that can be categorized according to their molecular weight and chemical structures<sup>[15]</sup>. Among them, CK19 is the least acidic, with a molecular weight of approximately 40 kDa, and is typically expressed in laminated epithelium<sup>[16]</sup>. The proliferative subtype of HCC that expresses CK19 is also referred to as biphenotypic HCC<sup>[5]</sup>. CK19 acts as a marker of bipotential progenitor cells capable of differentiating into both hepatocytes and biliary epithelial cells<sup>[17]</sup>. Approximately 10%-30% of HCC cases express CK19<sup>[18-20]</sup>. When present, CK19 expression in HCC is associated with pathological features of both HCC and cholangiocarcinoma, resulting in a poor prognosis<sup>[21]</sup>. Moreover, the combined assessment of pathological features and biomarkers such as CK19 has shown higher specificity and sensitivity in predicting metastatic recurrence in HCC<sup>[22]</sup>.

CK19 promotes epithelial-mesenchymal transition (EMT), thereby enhancing invasiveness and metastatic potential by downregulating E-cadherin and upregulating mesenchymal markers such as vimentin and N-cadherin<sup>[13]</sup>. It also identifies a subset of tumor-initiating cells co-expressing EpCAM, which display enhanced self-renewal, chemoresistance, and tumorigenicity<sup>[23,24]</sup>. Moreover, CK19-positive HCC activates the transforming growth factor-beta (TGF- $\beta$ ) pathway, which contributes to fibrosis, EMT, and

immunosuppression in later tumor stages<sup>[25]</sup>. Dysregulation of the Wnt/ $\beta$ -catenin pathway further supports proliferation, dedifferentiation, and the maintenance of cancer stem cell traits<sup>[26]</sup>. The Notch signaling pathway is also upregulated in CK19-positive tumors, acting synergistically with EpCAM to sustain a progenitor-like, therapy-resistant state<sup>[27]</sup>. Collectively, the EMT, TGF- $\beta$ , Wnt/ $\beta$ -catenin, Notch, and EpCAM pathways synergize to promote the aggressive, stem-like, and treatment-refractory characteristics of CK19-positive HCC, accounting for its association with microvascular invasion, early recurrence, chemoresistance, and poor prognosis<sup>[28]</sup>. CK19 expression is recognized as a marker of poor prognosis not only in HCC but also in lung, thyroid, colon, breast, and prostate cancers<sup>[29,30]</sup>. A recent study by Jia *et al.* reported CK19 in 100% of the biliary mucinous cystic neoplasms evaluated<sup>[31]</sup>.

Targeted therapies such as sorafenib, lenvatinib, and donafenib have shown clinical efficacy by disrupting key signaling pathways commonly activated in CK19-positive tumors. Sorafenib, a multikinase inhibitor, targets Rapidly Accelerated Fibrosarcoma (RAF), Vascular Endothelial Growth Factor Receptor (VEGFR), and Platelet-Derived Growth Factor Receptor (PDGFR), thereby inhibiting tumor proliferation via the RAF/Mitogen-activated protein kinase (MEK)/Extracellular Signal-Regulated Kinase (ERK) pathway and suppressing angiogenesis<sup>[32]</sup>. CK19-positive HCCs are often hypervascular, making them potentially responsive to VEGFR inhibition<sup>[20]</sup>. Lenvatinib exhibits broader activity, targeting VEGFR1-3, FGFR1-4, PDGFR $\alpha$ , RET, and KIT. It also inhibits the Fibroblast Growth Factor (FGF) pathway, which plays a role in the growth of stem-like, CK19-positive cells<sup>[33]</sup>. Through dual inhibition of VEGF and FGF signaling, lenvatinib may more effectively control tumor angiogenesis and cell proliferation. Its consistent therapeutic efficacy across various liver disease etiologies, including MAFLD, supports its use in CK19-positive HCC, regardless of underlying hepatic pathology<sup>[34]</sup>. Donafenib, a novel derivative of sorafenib, shares similar molecular targets but provides improved safety and tolerability, particularly in Asian populations, making it a suitable alternative for CK19-positive HCC patients requiring long-term treatment<sup>[35]</sup>. The introduction of molecular targeted agents such as lenvatinib, alongside immune checkpoint inhibitors like nivolumab and pembrolizumab, has improved survival outcomes. Notably, although the combination of atezolizumab and bevacizumab has demonstrated superior efficacy over sorafenib, establishing a new first-line treatment standard for advanced HCC<sup>[36]</sup>, its effectiveness in CK19-positive HCC remains to be fully confirmed.

CK19-positive HCC is aggressive, poorly differentiated, resistant to therapy, and highly recurrent, yet remains an underrecognized subtype. This makes it a compelling area for further investigation. The aim of this study is to elucidate the prognostic significance of CK19-positive HCC and evaluate the therapeutic efficacy of targeted therapy in these patients.

## MATERIALS AND METHODS

### Data collection

Clinical records of 310 patients diagnosed with HCC at the First Affiliated Hospital with Nanjing Medical University between 2010 and 2020 were retrospectively collected. The initial clinical diagnosis of HCC was based on two criteria: the presence of a liver lesion > 10 mm in diameter and an  $\alpha$ -fetoprotein (AFP) level  $\geq$  20 ng/mL<sup>[37]</sup>. The diagnosis was pathologically confirmed by two independent pathologists based on biopsy samples. The study was conducted in accordance with institutional ethical guidelines and the principles outlined in the Declaration of Helsinki.

### Inclusion and exclusion criteria

Patients who underwent curative resection, TACE, or neoadjuvant immunotherapy were considered for inclusion. Forty patients were excluded based on the following criteria: recurrent HCC (n = 20), presence of other malignancies (n = 11), distant metastasis confirmed either preoperatively or within one month

postoperatively (n = 3), or palliative resection (n = 6). After applying these criteria, a total of 270 patients were included in the final analysis.

### Detection of CK19 expression

Following surgical resection, tumor samples were subjected to immunohistochemical analysis to evaluate CK19 expression. Formalin-fixed, paraffin-embedded tissue sections were stained with anti-CK19 antibodies. CK19 expression was assessed via light microscopy. Tumors were classified as CK19-positive if  $\geq 5\%$  of cells showed immunoreactivity; otherwise, they were considered CK19-negative.

### Targeted therapy regimen

Targeted therapy was administered to patients with advanced, unresectable tumors. Among the 270 included patients, 56 patients received targeted therapy, 15 of them were CK19-positive. The therapeutic agents used were lenvatinib, sorafenib, and donafenib. Lenvatinib was given at 8 mg once daily for patients weighing less than 60 kg, and at 12 mg once daily for those weighing  $\geq 60$  kg. Sorafenib was administered at 400 mg twice daily, and donafenib at 200mg twice daily. Treatment continued until the patient no longer derived clinical benefit.

### Patient grouping

The 270 patients were categorized into two groups based on CK19 expression: 102 were CK19-positive and 168 were CK19-negative.

### Statistical analysis

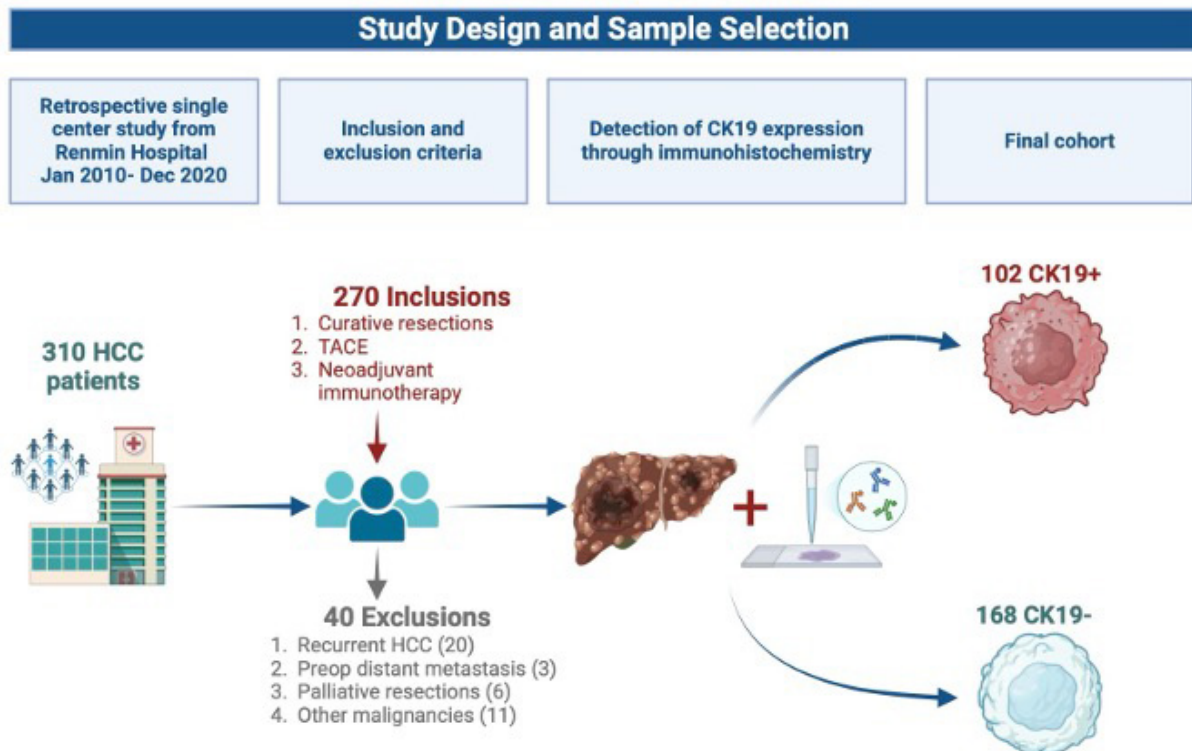
Patient characteristics were classified as either continuous or categorical variables. Continuous variables were summarized using medians and interquartile ranges (IQRs), and categorical variables as frequencies and percentages. The Student's *t*-test or Mann-Whitney *U* test was used to compare continuous variables, while the Chi-square test or Fisher's exact test was applied to categorical variables. Differences in recurrence-free survival (RFS) and overall survival (OS) between the two groups were assessed using the log-rank test. RFS was defined as the time to tumor recurrence, and OS as the time to death. Survival curves were plotted using the Kaplan-Meier method. Cox proportional hazards models were employed for univariate and multivariate analyses using a backward stepwise selection approach. Predicted survival plots were constructed based on multivariable Cox regression. A subgroup analysis of RFS was also performed based on factors such as age, gender, cirrhosis status, etiology, Child-Pugh grade, tumor size, tumor number, vascular invasion, and Edmondson-Steiner grade. A two-tailed *P*-value  $< 0.05$  was considered statistically significant.

All statistical analyses were performed using R software (version 4.2.2, R Project for Statistical Computing; <http://www.r-project.org>) and Stata (version 17.0).

## RESULTS

### Patient population and characteristics

A total of 310 patients diagnosed with HCC were initially included in the analysis. After applying the exclusion criteria (outlined in [Figure 1](#)), 40 patients were excluded, resulting in a final cohort of 270 patients, comprising 102 CK19-positive and 168 CK19-negative individuals. The median age of the study population was 58 years (interquartile range: 50-66 years), with a predominance of male patients (n = 211, 78.1%).



**Figure 1.** Flowchart of patient selection following the application of inclusion and exclusion criteria. HCC: Hepatocellular carcinoma.

Among the CK19-positive group, 79.4% were male. CK19-negative expression was identified in 168 patients. A comparative analysis of clinicopathological features between the CK19-positive and CK19-negative groups, as presented in Table 1, revealed statistically significant differences in several parameters. CK19-positive patients were more likely to be asymptomatic at presentation ( $P = 0.014$ ), to have moderately to poorly differentiated Edmondson-Steiner histological grades ( $P < 0.001$ ), to show elevated AFP levels ( $P = 0.0001$ ), and to exhibit a higher incidence of microvascular invasion ( $P < 0.010$ ). However, no significant association was observed between CK19 expression and hepatitis B or C infection, nor with the presence of liver cirrhosis in this cohort.

#### Comparison of OS and RFS pattern between CK19-negative and CK19-positive groups

RFS and OS were compared between CK19-positive and CK19-negative HCC patients using the log-rank test, with the results summarized in Table 2. CK19-positive patients exhibited significantly higher recurrence rates across all time points: 64.2% at 1 year, 44.5% at 3 years, 36.2% at 5 years, and 34.0% at 10 years. Moreover, the median survival time following recurrence in CK19-positive patients was approximately three times shorter than that of their CK19-negative counterparts, indicating a more aggressive disease course.

Similarly, OS was consistently worse in the CK19-positive group, with survival rates of 82.2% at 1 year, 60.1% at 3 years, 49.0% at 5 years, and 44.4% at 10 years. The median OS for CK19-negative patients was nearly twice as long as that of CK19-positive patients, further underscoring the adverse prognostic implications of CK19 expression in HCC.

**Table 1. Baseline characteristics of CK19-negative and -positive patient groups**

Characteristics	All patients (n = 270)	CK19-Negative (n = 168)	CK19-Positive (n = 102)	P-value
Median age (years)	58 (50-66)	58.5 (51.0-67.5)	57.0 (49.0-65.0)	0.325
Gender				0.695
Male	211 (78.1)	130 (77.4)	81 (79.4)	
Female	59 (21.9)	38 (22.6)	21 (20.6)	
Comorbid illness				0.133
Absent	181 (67)	107 (63.7)	74 (72.5)	
Present	89 (33)	61 (36.3)	28 (27.5)	
Hypertension	78 (28.9)	54 (32.1)	24 (23.5)	0.130
Diabetes mellitus	37 (13.7)	22 (13.1)	15 (14.7)	0.709
Asymptomatic HCC				0.014
Yes	173 (64.1)	117 (69.6)	56 (54.9)	
No	97 (35.9)	51 (30.4)	46 (45.1)	
Smoking				0.625
Yes	68(25.2)	44(26.2)	24(23.5)	
No	202(74.8)	124(73.8)	78(76.5)	
Alcohol consumption				0.814
Yes	51(18.9)	31(18.4)	20(19.6)	
No	219(81.1)	137(81.6)	82(80.4)	
AFP	14.5(3.5-380.2)	6.6(3.2-111.8)	130.6(6.4-1210)	0.0001
Etiology				0.926
Hepatitis B or C virus	195 (72.2)	121 (72)	74 (72.5)	
Others	75 (27.8)	47 (28)	28 (27.5)	
Cirrhosis				0.980
Yes	193 (71.5)	120 (71.4)	73 (71.6)	
No	77 (28.5)	48 (28.6)	29 (28.4)	
Child-Pugh class				0.133
A	258 (95.6)	163 (97)	95 (93.1)	
B	12 (4.4)	5 (3)	7 (6.9)	
Tumor size (cm)	4.0 (2.5-7.0)	4.0 (2.5-6.8)	4.0 (2.5-7.0)	0.842
Tumor number				0.089
Solitary	213 (78.9)	127 (75.6)	86 (84.3)	
Multiple	57 (21.1)	41 (24.4)	16 (15.7)	
Macrovascular invasion				0.075
Absent	244 (90.4)	156 (92.9)	88 (86.3)	
Present	26 (9.6)	12 (7.1)	14 (13.7)	
Extracapsular invasion				0.687
Absent	251 (93)	157 (93.5)	94 (92.2)	
Present	19 (7)	11 (6.5)	8 (7.8)	
Lymph node metastasis				0.780
Absent	261 (96.7)	162 (96.4)	99 (97.1)	
Present	9 (3.3)	6 (3.6)	3 (2.9)	
Edmondson-Steiner grade				< 0.001
Well	121 (44.8)	88 (52.4)	33 (32.4)	
Moderate	73 (27)	47 (28)	26 (25.5)	
Poor	76 (28.1)	33 (19.6)	43 (42.2)	
Microscopic vascular invasion				0.010
Absent	201 (74.4)	134 (79.8)	67 (65.7)	
Present	69 (25.6)	34 (20.2)	35 (34.3)	



Resection margin adjacent to tumor				0.506
No	235 (87)	148 (88.1)	87 (85.3)	
Yes	35 (13)	20 (11.9)	15 (14.7)	
TNM 8th stage				0.553
IA/IB	155 (57.4)	100 (59.5)	55 (53.9)	
II/IIIA	65 (24.1)	40 (23.8)	25 (24.5)	
IIIB/IVA	50 (18.5)	28 (16.7)	22 (21.6)	
Resection				0.451
Major hepatectomy	54 (20)	36 (21.4)	18 (17.6)	
Minor hepatectomy	216 (80)	132 (78.6)	84 (82.4)	
HCC-related local treatment				0.262
Absent	257 (95.2)	158 (94)	99 (97.1)	
Present	13 (4.8)	10 (6)	3 (2.9)	

CK19: Cytokeratin 19; HCC: hepatocellular carcinoma; AFP:  $\alpha$ -fetoprotein.

**Table 2. Survival outcomes in CK19-negative vs. CK19-positive patients**

	Events (%)	MST (95%CI)	1 year-	3 year-	5 year-	10 year-	Log-rank $\chi^2$	P-value
<b>RFS</b>								
Total	156 (57.8%)	43.1 (34.1-60.8)	72.3%	56.0%	45.4%	28.5%		
CK19 (-)	94 (56.0%)	60.3 (42.5-70.6)	77.3%	62.9%	51.1%	26.8%	4.60	0.032
CK19 (+)	62 (60.8%)	22.1 (14.1-37.8)	64.2%	44.5%	36.2%	34.0%		
<b>OS</b>								
Total	113 (41.9%)	90.2 (69.9-114.1)	88.1%	71.0%	60.9%	40.9%		
CK19 (-)	64 (38.1%)	101.6 (82.2-NA)	91.6%	77.6%	67.9%	40.5%	7.69	0.006
CK19 (+)	49 (48.0%)	56.4 (30.2-NA)	82.2%	60.1%	49.0%	44.4%		

\*MST: median survival time, in months. CK19: Cytokeratin 19; RFS: recurrence-free survival; OS: overall survival.

### Predictors of RFS and OS

Multivariate Cox proportional hazards analysis, adjusted for relevant covariates, identified CK19 expression as an independent negative prognostic factor for both RFS and OS. CK19 positivity was associated with a 43% increased risk of recurrence [HR 1.43, 95%CI (1.02-2.01),  $P = 0.041$ ] and a 60% higher risk of death [HR 1.60, 95%CI (1.07-2.40),  $P = 0.023$ ], as detailed in [Table 3](#) and illustrated in [Figure 2](#). These findings suggest a significant association between CK19 expression and both increased tumor recurrence and reduced survival outcomes.

In the univariate analysis of RFS, several clinicopathologic factors were significantly associated with poorer outcomes, including CK19 positivity, female sex, hepatitis infection, cirrhosis, multiple tumor nodules, tumor size > 5 cm, macrovascular invasion, microvascular invasion, extracapsular invasion, lymph node metastasis, and moderate to poor Edmondson-Steiner grade [[Table 3](#)]. In the multivariate analysis, CK19 positivity, cirrhosis, multiple tumors, tumor size > 5 cm, and moderate to poor tumor differentiation remained independent predictors of poor RFS.

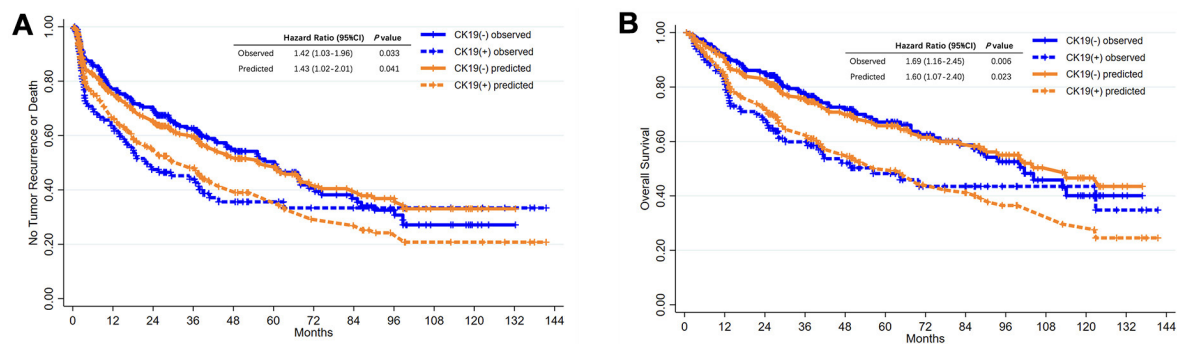
Similarly, the univariate analysis of OS identified CK19 positivity, asymptomatic presentation, Child-Pugh class B, multiple tumors, tumor size > 5 cm, macrovascular invasion, microvascular invasion, extracapsular invasion, lymph node metastasis, moderate to poor Edmondson-Steiner grade, and major hepatic resection as significant adverse prognostic factors. Multivariate analysis further confirmed that CK19 positivity, Child-Pugh class B, multiple tumors, larger tumor size (> 5 cm), macrovascular invasion, microvascular

**Table 3. Multivariate analysis of predictors for recurrence-free survival and overall survival**

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	P	Hazard ratio (95%CI)	P
<b>RFS analyzes</b>				
Age ( $\geq 60$ vs. $< 60$ yr)	0.79 (0.57-1.08)	0.142		
gender (female vs. male)	0.56 (0.36-0.86)	0.009		
Comorbid illness (present vs. absent)	0.75 (0.53-1.06)	0.105		
Asymptomatic HCC (no vs. yes)	1.34 (0.97-1.85)	0.071		
Etiology (hepatitis vs. others)	1.59 (1.09-2.33)	0.016		
Cirrhosis (yes vs. no)	1.50 (1.03-2.19)	0.035	1.82 (1.23-2.67)	0.002
Child-Pugh class (B vs. A)	1.57 (0.77-3.22)	0.214		
Tumor size ( $> 5$ vs. $\leq 5$ cm)	1.95 (1.42-2.68)	$< 0.001$	1.91 (1.37-2.68)	$< 0.001$
Tumor no. (multiple vs. solitary)	1.71 (1.20-2.46)	0.003	1.63 (1.12-2.37)	0.011
Macrovascular invasion (present vs. absent)	3.96 (2.46-6.37)	$< 0.001$	2.60 (1.56-4.34)	$< 0.001$
Extracapsular invasion (present vs. absent)	2.34 (1.34-4.08)	0.003		
Lymph node metastasis (yes vs. no)	3.41 (1.67-6.97)	0.001		
Edmondson-Steiner grade, well				
Moderate vs. well	1.75 (1.19-2.58)	0.004	1.56 (1.06-2.30)	0.025
Poor vs. well	2.41 (1.65-3.52)	$< 0.001$	1.97 (1.32-2.94)	0.001
Microscopic vascular invasion (present vs. absent)	2.40 (1.69-3.40)	$< 0.001$		
Resection margin adjacent to tumor (yes vs. no)	1.17 (0.73-1.87)	0.519		
Resection (major vs. minor hepatectomy)	0.70 (0.48-1.02)	0.061		
HCC-related local treatment (present vs. absent)	1.45 (0.74-2.85)	0.285		
CK19 (positive vs. negative)	1.42 (1.03-1.96)	0.033	1.43 (1.02-2.01)	0.041
<b>OS analyzes</b>				
Age ( $\geq 60$ vs. $< 60$ yr)	0.85 (0.58-1.24)	0.400		
Gender (female vs. male)	0.66 (0.40-1.07)	0.093		
Comorbid illness (present vs. absent)	0.81 (0.54-1.24)	0.338		
Asymptomatic HCC (no vs. yes)	1.81 (1.25-2.63)	0.002		
Etiology (hepatitis vs. others)	1.57 (1.00-2.46)	0.051		
Cirrhosis (yes vs. no)	1.22 (0.79-1.87)	0.366		
Child-Pugh class (B vs. A)	2.76 (1.33-5.70)	0.006	2.34 (1.07-5.13)	0.033
Tumor size ( $> 5$ vs. $\leq 5$ cm)	2.60 (1.79-3.76)	$< 0.001$	2.16 (1.43-3.26)	$< 0.001$
Tumor no. (multiple vs. solitary)	1.84 (1.22-2.77)	0.004	1.70 (1.09-2.65)	0.019
Vascular invasion				
Microscopic invasion vs. absent	2.77 (1.70-4.52)	$< 0.001$	1.84 (1.09-3.08)	0.022
Macrovascular invasion vs. absent	7.84 (4.68-13.13)	$< 0.001$	3.16 (1.76-5.69)	$< 0.001$
Extracapsular invasion (present vs. absent)	3.45 (1.91-6.24)	$< 0.001$		
Lymph node metastasis (yes vs. no)	4.47 (2.25-8.89)	$< 0.001$		
Edmondson-Steiner grade				
Moderate vs. well	2.89 (1.79-4.65)	$< 0.001$	2.59 (1.58-4.25)	$< 0.001$
Poor vs. well	4.02 (2.52-6.43)	$< 0.001$	2.72 (1.64-4.49)	$< 0.001$
Resection margin adjacent to tumor (yes vs. no)	1.21 (0.70-2.08)	0.500		
Resection (major vs. minor hepatectomy)	0.60 (0.39-0.91)	0.015		
HCC-related local treatment (present vs. absent)	1.06 (0.43-2.61)	0.894		
CK19 (positive vs. negative)	1.69 (1.16-2.45)	0.006	1.60 (1.07-2.40)	0.023

RFS: Recurrence-free survival; OS: overall survival; CK19: cytokeratin 19; HCC: hepatocellular carcinoma.





**Figure 2.** Survival curves from multivariate Cox regression for (A) recurrence-free survival and (B) overall survival.

invasion, and moderate to poor histological differentiation were independently associated with decreased OS.

### Subgroup analysis of RFS and OS

A sensitivity analysis of patient characteristics, illustrated in Figure 3, was conducted to validate the robustness of the covariate analyses. The results were consistent with the primary findings. No statistically significant differences in RFS or OS were observed between CK19-positive and CK19-negative patients in subgroups where the confidence intervals crossed the line of no effect. However, a general trend toward poorer outcomes was evident among CK19-positive patients compared to their CK19-negative counterparts. Elevated recurrence rates were observed in nearly all CK19-positive subgroups, with particularly pronounced effects in patients aged < 60 years, males, those with hepatitis B or C infection, Child-Pugh class A liver function, tumors > 5 cm in diameter, solitary tumors, vascular invasion (microscopic or macroscopic), and poor histological differentiation. Similarly, reduced overall survival was also more common among CK19-positive patients, especially within the same clinical subgroups: age < 60 years, male sex, viral hepatitis, preserved liver function (Child-Pugh A), large solitary tumors, vascular invasion (both microscopic and macroscopic), poor differentiation, and underlying cirrhosis.

### RFS and OS according to tumor, node, metastasis tumor stage

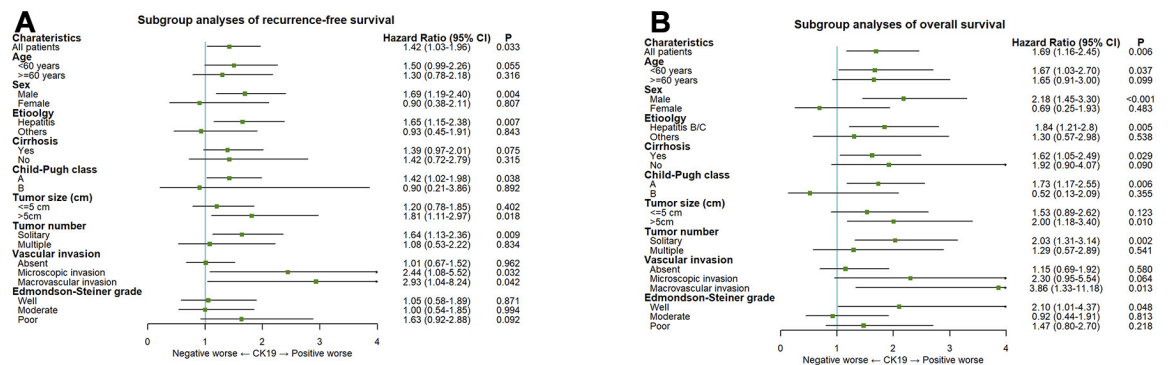
Figure 4 presents RFS and OS stratified by tumor, node, metastasis (TNM) stages IA/IB, II/IIIA, and IIIB/IVA in both CK19-positive and CK19-negative patients. A significantly poorer prognosis was observed in CK19-positive patients at the advanced stages, namely TNM stage IIIB/IVA (panels C and F).

### Effect of targeted therapy in CK19-positive HCC

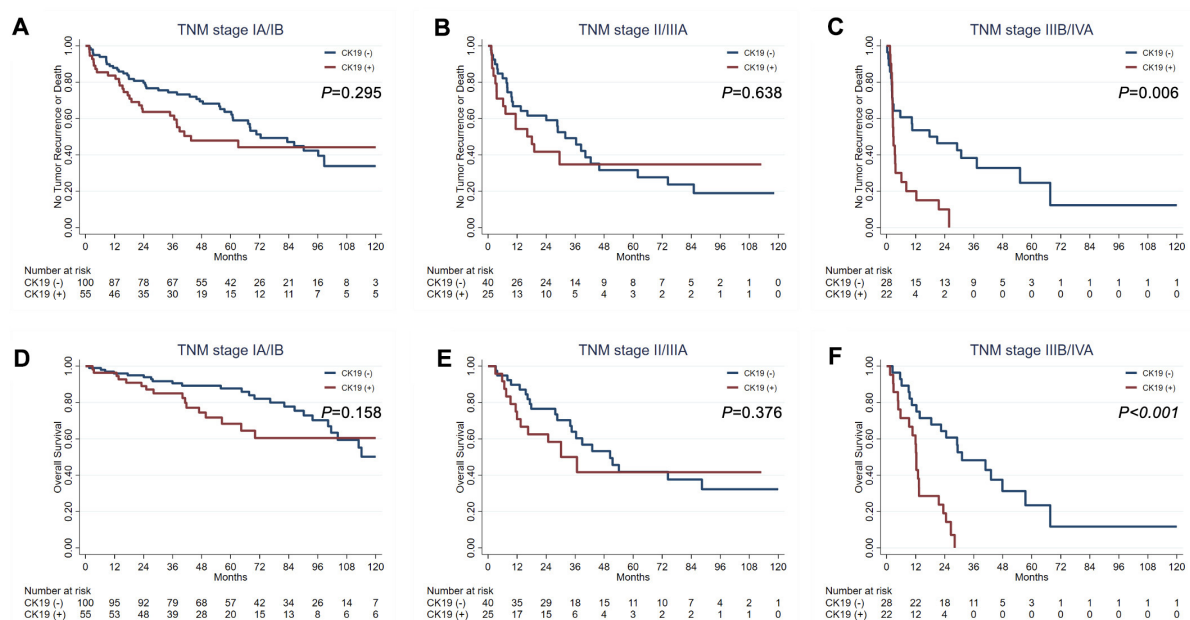
Among the 270 patients included in the study, 56 received postoperative targeted therapy, 15 of whom were CK19-positive. Figure 5 shows that there was no statistically significant relation between CK19 positivity and administration of targeted therapy ( $P = 0.064$ ). Furthermore, survival outcomes [RFS ( $P = 0.8888$ ) and OS ( $P = 0.1721$ )] were not statistically significant between those who received targeted therapy and those who did not.

## DISCUSSION

The present study is consistent with previous reports suggesting that CK19-expressing HCC represents a biologically aggressive subtype<sup>[38]</sup>. All CK19-positive patients with end-stage disease (TNM stage IIIB/IVA) experienced tumor recurrence or death within two years after curative resection. Similarly, Uenishi *et al.* reported that 73% of CK19-positive patients developed early postoperative recurrences<sup>[39]</sup>. Our findings further show that a moderate to poor Edmondson-Steiner grade is significantly associated with reduced OS



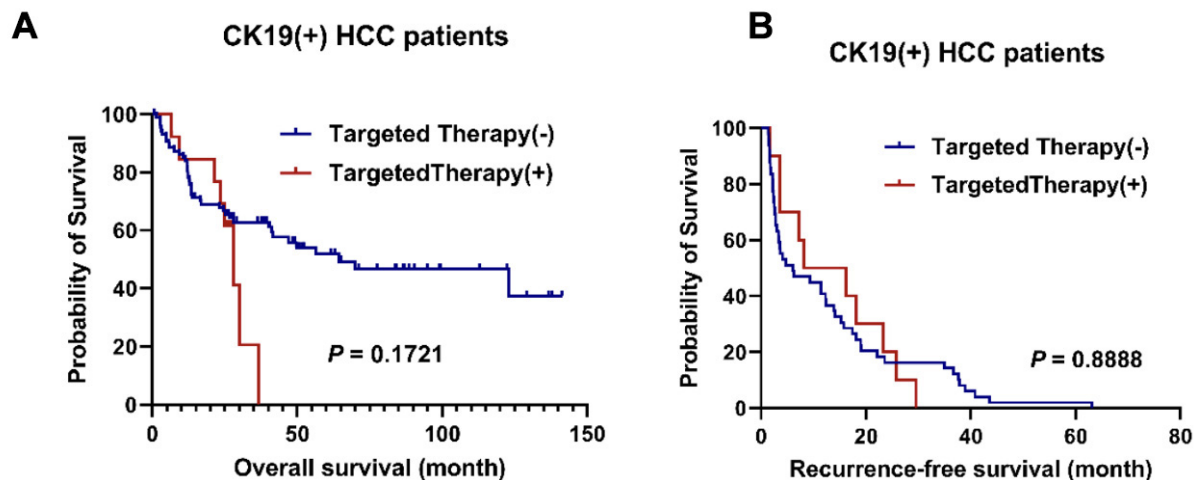
**Figure 3.** Subgroup analyses of recurrence-free survival (A) and overall survival (B). Shared clinicopathological characteristics of CK19-positive and CK19 patients were thoroughly examined. CK19: Cytokeratin 19.



**Figure 4.** Kaplan-Meier analysis of recurrence-free survival (A-C) and overall survival (D-F) by TNM stage and CK19 status. TNM: Tumor, node, metastasis; CK19: cytokeratin 19.

and RFS in CK19-positive patients, corroborating earlier studies indicating that poorly differentiated HCC more frequently expresses CK19<sup>[39]</sup>. Subgroup analyses revealed that coexisting hepatitis B or C infection was associated with worse outcomes in both RFS and OS. CK19-positive HCC patients with hepatitis B have been shown to exhibit higher early recurrence rates and poorer prognosis<sup>[38,40,41]</sup>. The relationship among AFP, CK19, and HCC remains complex. AFP-positive HCC patients tend to present with larger tumors, more advanced stages, elevated HBV markers, and increased liver enzyme levels and blood cell counts compared to AFP-negative patients<sup>[42]</sup>.

Macrovascular invasion, tumor multiplicity, and increased tumor size are well-established predictors of advanced disease and poor prognosis in HCC<sup>[43-46]</sup>. These features reflect aggressive tumor biology and are frequently associated with higher recurrence rates and reduced OS. CK19 expression has increasingly been recognized as a marker of such biological aggressiveness. Several studies have demonstrated that CK19-positive tumors are significantly more likely to exhibit microvascular invasion - a key



**Figure 5.** The OS (A) and RFS (B) in CK19-positive patients that received targeted therapy postoperatively. OS: Overall survival; RFS: recurrence-free survival; CK19: cytokeratin 19.

histopathological hallmark of early metastatic potential and an independent predictor of recurrence and mortality<sup>[39,47]</sup>. In our cohort, gross tumor analyses revealed a higher prevalence of microvascular invasion among CK19-positive patients, aligning with earlier reports of enhanced vascular infiltration and invasiveness in this subtype<sup>[5]</sup>. Additionally, our data indicate that CK19 positivity was associated with a 1.43-fold increase in recurrence risk and a 1.60-fold reduction in survival probability, emphasizing its prognostic significance. These findings support the growing consensus that CK19 is not merely a phenotypic marker but a clinically meaningful biomarker indicative of adverse outcomes.

Although alcohol consumption and cigarette smoking are well-established etiological factors for HCC, their specific associations with CK19-positive HCC remain poorly defined. Chronic alcohol intake contributes to HCC pathogenesis primarily through mechanisms such as liver cirrhosis, oxidative stress, and DNA damage. Epidemiological data suggest that alcohol-related cirrhosis accounts for approximately 30%-40% of HCC cases in Western populations<sup>[48,49]</sup>. Similarly, cigarette smoking is an independent risk factor for HCC, with meta-analyses showing a 51% increased risk in current smokers and a 12% increase in former smokers compared to non-smokers<sup>[50]</sup>. The carcinogenicity of tobacco is partly mediated by the activation of oncogenic pathways, such as p53 mutations, and synergistic interactions with viral hepatitis. However, limited data exist regarding the influence of alcohol and smoking on CK19-positive HCC. While CK19-positive tumors often exhibit microvascular invasion and poor differentiation, current studies - including our own - have not conclusively linked these exposures to CK19 expression. Most investigations into CK19 have focused on molecular or histological features rather than modifiable risk factors, revealing a critical gap in understanding the environmental and lifestyle contributors to this aggressive HCC subtype. Further prospective studies are warranted to determine whether alcohol and smoking influence the development or molecular phenotype of CK19-positive HCC.

The benefits of targeted therapy for CK19-positive HCC remain to be confirmed in larger cohorts. In addition to the financial burden, patients may suffer from increased side effects if the treatment proves ineffective. Previous studies have reported that both chemotherapy and targeted therapy yield limited benefit in CK19-positive HCC<sup>[51]</sup>. Given the complexity of these tumors and their involvement in multiple activated signaling pathways, combination therapies - such as targeted agents with immunotherapies (e.g., anti-PD-1/PD-L1 antibodies) or locoregional approaches (TACE or radiotherapy) - may enhance outcomes

by simultaneously addressing both intrinsic tumor resistance and the tumor microenvironment. Our findings support the need for future studies exploring tailored combination strategies based on the molecular profile of CK19-positive HCC. Regorafenib has shown promise as a targeted agent in CK19-positive HCC and warrants further investigation in larger trials<sup>[52]</sup>. Wu *et al.* reported that adjuvant TACE may only delay recurrences but can prolong survival in CK19-positive patients<sup>[53]</sup>.

Lymph node metastasis in HCC remains relatively rare, with reported incidences ranging from 1.7% to 7.5%<sup>[39,54]</sup>. Patients with lymph node involvement generally face poor outcomes despite undergoing lymphadenectomy, with a reported 5-year survival rate of only 31%<sup>[55,56]</sup>. Current European and American guidelines do not recommend routine lymphadenectomy unless there is strong radiological or intraoperative evidence, particularly in aggressive subtypes with suspected extrahepatic spreads. In our study, no significant correlation was observed between CK19 expression and lymph node metastasis. However, Zhuang *et al.* reported a significantly higher incidence (27.7%) of lymph node metastasis in CK19-positive patients compared to 5.6% in CK19-negative patients<sup>[57]</sup>. Moreover, CK19-positive patients with lymph node involvement had significantly worse prognoses<sup>[57]</sup>.

This study highlights the clinical relevance of CK19 expression in four key domains: (1) Prognostic stratification - CK19 status enables early risk assessment and identification of aggressive tumor subtypes prior to overt clinical progression; (2) Therapeutic decision making - CK19 positivity indicates potential resistance to tyrosine kinase inhibitors (TKIs), suggesting the need for alternative or combination regimens; (3) Biomarker-driven therapies - CK19 serves as a foundation for targeted therapies focused on stemness, EMT, and cholangiocytic differentiation. It may also be useful in identifying candidates for precision oncology trials; (4) Postoperative management - Due to the high recurrence risk, CK19-positive patients may benefit from intensified surveillance, earlier adjuvant or neoadjuvant therapy, and more accurate preoperative diagnostics. Semi-annual surveillance in cirrhotic patients has been shown to significantly improve early detection and access to curative treatments. A meta-analysis involving 15,158 patients demonstrated a 2.08-fold increase in early-stage HCC diagnosis and a 2.24-fold rise in curative therapy, leading to improved overall survival (OR 1.90).

The aggressive nature of CK19-positive HCC suggests that effective management may require multifaceted strategies. Combination approaches - including immunotherapy, targeted therapy, ablation, and resections - may improve outcomes. For instance, combining TACE with a Programmed Cell Death 1 (PD1) -inhibitor and Lenvatinib was shown to significantly prolong survival compared to TACE plus PD1 inhibition alone<sup>[58]</sup>. Despite resistance to several chemotherapies, regorafenib, which targets the STAT3/mitochondria axis, exhibits preferential efficacy against CK19-positive HCC<sup>[52]</sup>. In patient-derived xenograft models, regorafenib achieved an 80% tumor control rate in CK19-positive tumors, compared to 0% in CK19-negative cases<sup>[59]</sup>. However, mechanisms such as inflammation, hypoxia, and EMT may limit its effectiveness. Research is ongoing into enhancing regorafenib's efficacy through combination with immunotherapy techniques<sup>[60]</sup>.

The limitations of this study include population selection bias, retrospective design, endpoint definition inconsistencies, and non-uniform follow-up schedules. Although around 2,500 patients were diagnosed with HCC between 2010 and 2020, only 310 underwent immunohistochemical evaluation, and further exclusions reduced the cohort size. Multivariate Cox regression was employed to adjust for confounding variables, but the variability in follow-up duration remains a concern. Routine lymphadenectomy was not performed in CK19-positive patients, limiting conclusions regarding the association between CK19 expression and lymph node metastasis. Additionally, liver disease etiology was broadly classified as viral or

non-viral, preventing more nuanced subgroup analysis [e.g., MAFLD, alcohol-related liver disease(ALD)]. Future studies with more granular etiological data are necessary to fully understand how CK19 expression interacts with underlying liver conditions and affects treatment response.

Research in this area has evolved from focusing on prognostic evaluation to exploring mechanisms of CK19 action, therapeutic strategies, and non-invasive diagnostic tools. Given the aggressive behavior of CK19-positive HCC, early and accurate diagnosis using imaging modalities such as contrast-enhanced ultrasound (US)<sup>[61]</sup>, triphasic Computed Tomography (CT)<sup>[62]</sup>, Gadolinium ethoxylbenzylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced Magnetic Resonance Imaging (MRI)<sup>[63]</sup>, Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET)<sup>[64]</sup>, Magnetic Resonance Elastography (MRE)<sup>[65]</sup>, and deep learning radiomics based on Gadoteric Acid-Enhanced MRI<sup>[66]</sup> may significantly improve outcomes. Personalized treatment strategies and clinical trials are essential to developing evidence-based approaches for managing this subtype. Furthermore, elucidating the underlying causes of CK19 expression could inform preventive strategies.

## Conclusion

In summary, this study confirms that CK19-positive HCC is associated with a poorer prognosis than CK19-negative HCC. Patients with CK19-positive tumors are more likely to experience early recurrence and have shorter overall survival. CK19 expression can thus be considered an independent prognostic marker for predicting OS and RFS in HCC. Additionally, current adjuvant targeted therapies do not appear to confer a survival benefit in CK19-positive patients, emphasizing the need for novel and more effective therapeutic approaches.

## DECLARATIONS

### Authors' contributions

Study conceptualization: Khodabocus Z, Wang X, Mu X, Chen Z

Data curation and analysis: Khodabocus Z, Zhang H, Mu X

Manuscript drafting and editing: Khodabocus Z, Zhang H, Li D, Chen Z

Manuscript draft revision: all authors revised the manuscript for important intellectual content and approved the final version of the article.

### Availability of data and materials

The datasets used and analyzed in this study are available from the corresponding author upon reasonable request.

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### Conflicts of interest

All authors declared that there are no conflicts of interest

### Ethics approval and consent to participate

Ethics approval was provided by the institutional review board of the First Affiliated Hospital of Nanjing Medical University (Approval number: 2023-SRFA-096). Informed consent to participate was obtained from all participants.



## Consent for publication

Not applicable

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