Serum CA19-9 Level is Correlated with Liver Inflammation, Cirrhosis, and Poor Prognosis after Curative Resection in Hepatitis B-related Hepatocellular Carcinoma

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Abstract

Background: The relationship between serum carbohydrate antigen 19-9 (CA19-9) and hepatocellular carcinoma (HCC) is unclear. To explore the prognostic value of preoperative CA19-9 in α-fetoprotein (AFP)-positive and -negative HCC with hepatitis B virus (HBV) background (HBV-HCC).

Methods: We evaluated HBV-HCC patients who underwent curative resection (Cohort 1). Recurrence-free survival (RFS) and overall survival (OS) were assessed, and the clinicopathological characteristics were compared according to a CA19-9 cutoff of ≥ 39 U/ml. Immunohistochemical staining of CA19-9 in HCC tumor tissue and background liver were quantified in another cohort of 216 patients with resected HCC (Cohort 2). Immunohistochemical staining or serum CA19-9 level was also compared between patients with intrahepatic cholangiocarcinoma (ICC) (Cohort 3).

Results: In Cohort 1, CA19-9 ≥ 39 U/ml was an independent risk factor for RFS (HR=1.507, 95% CI=1.087-2.091, p=0.014) and OS (HR=1.646, 95% CI=1.146-2.366, p=0.007) in both AFP-positive and AFP-negative patients. CA19-9 ≥ 39 U/ml was also associated with significantly higher incidence of macrovascular invasion compared with CA19-9 < 39 U/ml (23.0% vs. 7.2%, p=0.002), elevated aminotransferase and aspartate aminotransferase to platelet ratio index, and lower albumin. In contrast, no association was found between CA19-9 and systemic inflammation. CA19-9 expression was found exclusively in the background liver but not in HCC tumor cells. In contrast, tumor tissue was the main source of CA19-9 in ICC patients.

Conclusions: CA19-9 ≥ 39 U/ml was associated with lower OS and RFS in both AFP-positive and negative HCC patients. CA19-9 is secreted by the background liver but not by tumor cells. Thus, CA19-9 is a prognostic marker for liver inflammation and cirrhosis in HCC.

Background

Primary liver cancer is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer-related death worldwide [1]. In general, primary liver cancer is classified into two types as hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma, with HCC being more common, accounting for 75%-85% of all cases. However, mixed HCC-ICC and other rare types have also been reported. Alpha-fetoprotein (AFP) and carbohydrate antigen 19 – 9 (CA19-9) are the most commonly used biomarkers for HCC and ICC. A higher AFP level is associated with poor outcomes after curative resection or liver transplantation [2–4]. CA19-9, also known as sialyl Lewis-a, is mainly used as a biomarker for malignancies of the hepatobiliary tract and pancreas[5]. However, serum CA19-9 levels may also be elevated in gastric, esophageal, and colonic cancers and in a number of non-malignant conditions that are associated with jaundice [6]. The serum CA19-9 level is elevated in approximately 60% of cholangiocarcinoma patients and in 30% of HCC patients [7]. It is also frequently elevated in patients with combined HCC-cholangiocarcinoma. Elevated preoperative serum CA19-9 levels have been reported to be associated with worse survival in HCC patients who had undergone resection (> 27 U/mL) or liver

transplantation (> 100 U/ml) [8–10]. However, most of these studies mainly included patients with HCV-related HCC who underwent resection or transplantation, and the underlying mechanism by which CA19-9 influences prognosis remains unclear.

Thus, this study aimed to investigate the prognostic value of preoperative serum CA19-9 according to AFP status in HCC patients and in ICC patients with HBV background who underwent curative resection.

Methods

Patients and study design

We retrospectively evaluated three patient cohorts as follows. Cohort 1 involved 380 patients diagnosed with HCC at Tianjin Medical University Cancer Institute & Hospital (Tianjin, People's Republic of China) between 2012 and 2013. In this cohort, CA19-9 (+) was defined as serum CA19-9 ≥ 39 U/ml, whereas CA19-9 (-) was defined as serum CA19-9 < 39 U/ml, according to the upper limit of serum CA19-9 in our hospital. AFP (+) was defined as serum AFP > 20 ng/ml, whereas AFP (-) was AFP ≤ 20 ng/ml. Cohort 2 involved 216 patients with resected HCC in whom tissue microarray (TMA) samples were obtained. Patients with lymph node metastasis or distant metastasis were excluded to reduce the confounding factors. Cohort 3 included 136 ICC patients whose paraffin blocks were collected based on the availability of suitable formalin-fixed, paraffin-embedded tissue and complete clinicopathologic and follow-up data of the patients.

All the patients underwent curative resection for HCC, defined as complete macroscopic removal of the tumor. All tumors were staged according to the TNM classification system of International Union Against Cancer (8th edition) and the Barcelona Clinic Liver Cancer guidelines.

Demographic And Clinicopathological Factors

Demographic and clinicopathological factors including tumor factors, systemic inflammation factors, and liver factors were evaluated. Demographic factors included sex and age. Tumor factors included tumor size, number of tumor lesions, macroscopic vascular invasion (MaVI), microscopic vascular invasion (MiVI), intrahepatic metastasis, and tumor differentiation according to Edmondson's grade. Systemic factors included the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR). Liver factors included intraoperative detection of liver cirrhosis; alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (rGT), alkaline phosphatase (ALP), total bilirubin, and albumin levels; prothrombin time (PT); and the aspartate aminotransferase-to-platelet ratio index (APRI) as the parameter most closely related to liver cirrhosis and fibrosis in both chronic hepatitis B [11–13] and hepatitis C [14, 15]. NLR and PLR are both indicators of systemic inflammation and its relationship with the prognosis of several cancers has been identified [16–19].

Tma In Icc

We selected 158 consecutive patients with ICC who underwent surgical treatment at Tianjin Medical University Cancer Institute and Hospital between January 2012 and December 2017. Patients with combined HCC-CCA (i.e., HCC and ICC) were excluded. The specimens of all patients were reviewed by two independent pathologists (Y.B. and Z.F.L.) to confirm the diagnosis of ICC and for restaging according to the 8th edition of the 2017 American Joint Committee on Cancer staging system. Of the 158 patients, we excluded 28 because of loss to follow-up (n = 11), non-R0 resection (n = 14), death from postoperative complications (n = 1), and death from non-tumor-related causes (n = 2). Thus, 130 patients (Cohort 3) were eventually included for the comparison of clinical characteristics and survival analyses. The patients' formalin-fixed paraffin-embedded (FFPE) samples and hematoxylin-eosin (HE) staining slides from surgical specimens were then collected from the Pathology Department of Tianjin Medical University Cancer Institute and Hospital. TMA samples comprising 2-mm cores of FFPE tumor tissue were prepared for various staining procedures by selecting representative tumor areas and a typical paratumoral region from each case. The Medical Ethics Committee of Tianjin Medical University Cancer Institute and Hospital approved this study, and informed consent was obtained from all patients or their legal guardian.

Follow-up And Postoperative Treatment

After surgery, patients were routinely followed. Follow-up was censored on July 10, 2019, with the patients followed up for a median of 56.6 months. All patients were monitored prospectively according to serum AFP and carbohydrate antigen 19 – 9 levels and using abdomen ultrasonography every 2 months in the first year and every 3 months after the first year. Recurrence was confirmed using computed tomography and/or magnetic resonance imaging based on typical imaging appearance in the imaging scan and an elevated AFP level. The treatment modality after relapse varied among individuals.

Tma And Immunohistochemistry

TMAs were constructed as described previously [20]. TMAs sections were dewaxed and rehydrated in xylene and gradient ethanol, respectively. After antigen retrieval and endogenous peroxidase activity blocking, the slides were incubated with primary antibodies (4 °C for 14 h and 37 °C for 1 h) and HRP-conjugated secondary antibody (37 °C for 1 h) in turn. Then the sections were visualized with 3,3'-diaminobenzidine (ZLI-9017; Zhongshan Goldbridge) for 5 min and counterstained with Hematoxylin. Appropriate internal or external positive controls and negative controls were designed and used for each round [20]. The primary antibody used in the present study was anti-human CA19-9 (Zhongshan Company). Negative control slides omitting the primary antibodies were included in all assays. CA19-9 immunoreactivity was evaluated in a semiquantitative manner by evaluating both staining intensity and the percentage lcf immunopositive tumor cells.

Statistical analysis

Kaplan-Meier method and Log-rank test with survival curves were used in univariate analyses of cumulative survival. Multivariate analyses were based on the Cox proportional hazards regression model. For the comparison of individual variables, χ^2 tests and Student's t-tests were used as appropriate. All statistical analyses were performed using SPSS software (SPSS v22.0, Chicago, IL). A two-tailed P value of < 0.05 was considered statistically significant.

Results

CA19-9 was an independent risk factor for RFS and OS

In Cohort 1, the 1-, 3-, and 5-year overall survival (OS) rates for CA19-9 (+) patients were 80.3%, 37.7%, and 34.4%, respectively, and were 90.3%, 62.7%, and 51.4%, respectively, for the CA19-9 (-) patients (Fig. 1a). The 1-, 3-, and 5-year RFS rates for the CA19-9 (+) patients were 45.9%, 14.8%, and 13.1%, respectively, and were 67.1%, 40.4%, and 33.5%, respectively, for the CA19-9 (-) patients (Fig. 1b). The 1-, 3-, and 5-year OS rates for AFP (+) patients were 84.0%, 47.1%, and 38.3%, respectively, and were 94.2%, 72.4%, and 60.9%, respectively, for the AFP (-) patients (Fig. 1c). The 1-, 3-, and 5-year RFS for the AFP (+) patients were 54.9%, 29.1%, and 24.8%, respectively, and were 74.7%, 44.8%, and 37.4%, respectively, for the AFP (-) patients (Fig. 1d).

In multivariate analysis, tumor size > 5 cm, presence of MaVI, AFP > 20 ng/ml, and CA19-9 ≥ 39 U/ml were independent risk factors for RFS. Meanwhile, tumor size > 5 cm, presence of MaVI, AFP > 20 ng/ml, CA19-9 ≥ 39 U/ml, and albumin ≤ 35 g/L were independent risk factors for OS (Tables 1 and 2).

Table 1
Univariate and multivariate analysis for RFS in Cohort 1.

Recurrence-free Survival Variable	Comparison	Univariate P-value	Multivariate P-value	Hazard Ratio (95.0% CI)
Gender	Male vs. female	0.268		
Age	≤ 50 vs.>50 years	0.957		
Tumor size	≤ 5 vs.>5 cm	< 0.001	0.001	1.569(1.204−2.045)
number	Solitary vs. multiple	0.075		
MaVI	Yes vs. no	< 0.001	0.023	1.586(1.065−2.361)
Differentiation	I/II/III/IV	0.482		
MiVI	Yes vs. no	0.025		
IHM	Yes vs. no	0.022		
Cirrhosis	Yes vs. no	0.588		
HBeAg	Yes vs. no	0.142		
PT	≤ 11 vs.>11seconds	0.338		
AFP	≤ 20 vs.>20 ng/mL	< 0.001	0.012	1.373(1.071−1.759)
CA19-9	≥ 39 vs.<39 U/ml	< 0.001	0.014	1.507(1.087−2.091)
ALT	≤ 40 vs.>40 U/L	0.046		
AST	≤ 40 vs.>40 U/L	< 0.001		
Albumin	≤ 35 vs.>35 g/L	0.114		
NLR	≤ 5 vs. >5	0.019		
PLR	≤ 300 vs. >300	0.072		
TB	≤ 19 vs.>19 μmol/L	0.975		
ALP	≤ 130 vs. >130	0.210		
rGT	≤ 60 vs.>60 U/L	< 0.001		
HKLC	0/1/2/3	< 0.001	NA	
BCLC	A/B/C	< 0.001	NA	
Note: MaVI, Macroscopic Vascular Invasion.				
MiVI, Microscopic Vascular Invasion.				

Recurrence-free Survival	Comparison	Univariate	Multivariate	
Variable		P-value	P-value	Hazard Ratio (95.0% CI)
IHM, Intrahepatic Metastasis.				
HBeAg, Hepatitis B virus e Antigen.				
PT, Prothrombin Time.				
AFP, α-fetoprotein.				
CA19-9, Carbohydrate Antigen 19 – 9.				
ALT, Alanine Aminotransferase.				
AST, Aspartate Aminotransferase.				
ALP, Alkaline Phosphatase.				
rGT, Gamma-glutamyl Transpeptidase.				
TB, Total Bilirubin.				
APRI, Aspartate aminotransferase to Platelet Ratio Index.				
NLR, Neutrophil-to-Lymphocyte Ratio .				
PLR, Platelet-to-Lymphocyte Ratio.				
NA, Not Applicable.				

Table 2
Univariate and multivariate analysis for OS in Cohort 1.

Overall Survival Variable	Comparison	Univariate P-value	Multivariate P-value	Hazard Ratio (95.0% CI)
Gender	Male vs. female	0.222		
Age	≤ 50 vs.>50 years	0.246		
Tumor size	≤ 5 vs.>5 cm	< 0.001	< 0.001	1.931(1.430−2.607)
Number	Solitary vs. multiple	0.029		
MaVI	Yes vs. no	< 0.001	0.003	1.871(1.230−2.847)
Differentiation	I/II/III/IV	0.216		
MiVI	Yes vs. no	< 0.001		
IHM	Yes vs. no	0.002	0.009	1.483(1.104−1.992)
Cirrhosis	Yes vs. no	0.158		
HBeAg	Yes vs. no	0.798		
PT	≤ 11 vs.>11 seconds	0.615		
AFP	≤ 20 vs.>20 ng/mL	< 0.001	0.003	1.558(1.163−2.089)
CA19-9	≥ 39 vs.<39 U/ml	0.001	0.007	1.646(1.146−2.366)
ALT	≤ 40 vs.>40 U/L	0.142		
AST	≤ 40 vs.>40 U/L	< 0.001		
NLR	≤ 5 vs. >5	< 0.001		
PLR	≤ 300 vs. >300	0.004	0.029	2.920(1.118−7.624)
ALP	≤ 130 vs. >30	0.026		
Albumin	≤ 35 vs.>35 g/L	0.174		
rGT	≤ 60 vs.>60 U/L	< 0.001		
TB	≤ 19 vs.>19 μmol/L	0.056		
HKLC	0/1/2/3	0.011	NA	
BCLC	A/B/C	< 0.001	NA	
Note: MaVI, Macroscopic Vascular Invasion.				
MiVI, Microscopic Vascular Invasion.				

Overall Survival	Comparison	Univariate	Multivariate	
Variable		P-value	P-value	Hazard Ratio (95.0% CI)
IHM, Intrahepatic Metastasis.				
HBeAg, Hepatitis B virus e Antigen.				
PT, Prothrombin Time.				
AFP, α-fetoprotein.				
CA19-9, Carbohydrate Antigen 19 – 9.				
ALT, Alanine Aminotransferase.				
AST, Aspartate Aminotransferase.				
ALP, Alkaline Phosphatase.				
rGT, Gamma-glutamyl Transpeptidase.				
TB, Total Bilirubin.				
APRI, Aspartate aminotransferase to Platelet Ratio Index.				
NLR, Neutrophil-to-Lymphocyte Ratio .				
PLR, Platelet-to-Lymphocyte Ratio.				
NA, Not Applicable.				

Positive CA19-9 predicted worse prognosis in both AFP (+) and AFP (-) HCC patients

The 1-, 3-, and 5-year OS for patients with CA19-9 (-) and AFP (-) were 95.4%, 74.5%, 61.4%, respectively, whereas they were 83.3%, 50.0%, and 45.8%, respectively, for patients with CA19-9 (+) and AFP (-) (p < 0.05, Fig. 1e). The 1-, 3-, and 5-year RFS for patients with CA19-9 (-) and AFP (-) were 77.1%, 47.1%, and 39.2%, respectively, whereas they were 45.8%, 20.8%, and 16.7%, respectively, in CA19-9 (+) and AFP (-) patients (p < 0.05, Fig. 1f). These results showed that CA19-9 (+) predicted worse OS and RFS in AFP (-) patients.

The 1-, 3-, and 5-year OS for patients with CA19-9 (-) and AFP (+) were 84.7%, 52.8%, and 42.9%, respectively, and were 77.5%, 27.5%, and 22.5%, respectively, for patients with CA19-9 (+) and AFP (+) (p < 0.05, Fig. 1e). The 1-, 3-, and 5-year RFS for patients with CA19-9 (-) and AFP (+) were 58.9%, 34.4%, and 28.8%, respectively (p < 0.05, Fig. 1f), whereas they were 40.0%, 10.0%, and 7.5%, respectively, for patients with CA19-9 (+) and AFP (+) (p < 0.05, Fig. 1f). These results indicate that CA19-9 (+) predicted worse OS

and RFS in AFP (+) patients. In summary, CA19-9 (+) predicted worse OS and RFS in both AFP (+) and AFP (-) HCC patients.

CA19-9 was associated with higher incidence of MaVI and a trend toward multiple tumors

CA19-9 was not associated with tumor size (6.1 ± 4.8 cm vs. 5.6 ± 3.8 cm, p = 0.404), MiVI (62.3% vs. 54.9%, p = 0.225) and AFP (5488.9 ± 28616.1 ng/ml vs. 5401.4 ± 40162.5 ng/ml, p = 0.987). However, CA19-9 was related to higher incidence of MaVI (23.0% vs. 7.2%, p = 0.002), and a trend toward more multiple tumors with marginal significance (23.0% vs. 13.8%, p = 0.068) (Table 3).

Table 3
Comparison of clinicopathological factors between patients with CA19-9 (+) and CA19-9 (-).

Cohort 1. Variable	CA 19 − 9 < 39 U/ml (n = 319)	CA 19 − 9 ≥ 39 U/ml (n = 61)	P-value
Gender (Male/Female)	255/64 (78.0%)	48/13 (78.7%)	0.824
Age (year) (Mean ± SD)	55.4 ± 10.6	58.4 ± 10.4	0.048
Tumor size (Mean ± SD)	5.6 ± 3.8	6.1 ± 4.8	0.404
Number (Solitary vs. multiple)	275/44 (13.8%)	47/14 (23.0%)	0.068
MaVI (Yes vs. no)	23/296 (7.2%)	12/49 (23.0%)	0.002
MiVI (Yes vs. no)	175/144 (54.9%)	38/22 (62.3%)	0.225
IHM (Yes vs. no)	111/208 (34.8%)	24/36 (39.3%)	0.440
AFP (ng/mL) (Mean ± SD)	5401.4 ± 40162.5	5488.9 ± 28616.1	0.987
HBeAg(Yes vs. no)	45/274	12/49	0.265
Cirrhosis (Yes vs. no)	179/140 (56.1%)	43/18 (70.5%)	0.037
APRI (Mean ± SD)	0.72 ± 0.96	1.53 ± 1.61	P < 0.001
Ascites (Yes vs. no)	32/287	7/54	0.733
rGT (Mean ± SD)	81.4 ± 99.4	147.1 ± 162.4	0.003
ALP (Mean ± SD)	95.7 ± 52.9	117.5 ± 70.2	0.006
ALT (U/L) (Mean ± SD)	43.9 ± 59.5	75.4 ± 77.3	0.004
AST (U/L) (Mean ± SD)	41.5 ± 46.0	75.1 ± 69.0	< 0.001
PT (seconds) (Mean ± SD)	11.3 ± 1.2	12.1 ± 1.0	< 0.001
Albumin (g/L) (Mean ± SD)	42.0 ± 5.3	39.8 ± 5.5	0.002
TB (μmol/L) (Mean ± SD)	18.5 ± 9.8	26.4 ± 37.8	0.109
NLR (Mean ± SD)	2.3 ± 1.5	2.1 ± 1.4	0.388
PLR (Mean ± SD)	134.3 ± 368.7	95.5 ± 47.8	0.412

CA19-9 was associated with more severe liver cirrhosis and liver inflammation but not with systemic inflammation

Comparison of clinicopathological factors between CA19-9 (+) and CA19-9(-) patients revealed that CA19-9 (+) patients tend to be older (mean age: 58.4 ± 10.4 years vs. 55.4 ± 10.6 years, p = 0.048), have higher incidence of liver cirrhosis (70.5% vs. 56.1%, p = 0.037), higher APRI (1.53 ± 1.61 vs. 0.72 ± 0.96, p < 0.001), longer PT (12.1 ± 1.0 vs. 11.3 ± 1.2, p < 0.001), elevated ALT (75.4 ± 77.3 U/L vs. 43.9 ± 59.5 U/L, p = 0.004), elevated AST (75.1 ± 69.0 U/L vs. 41.5 ± 46.0 U/L, p < 0.001), increased rGT (147.1 ± 162.4 U/L vs. 81.4 ± 99.4 U/L, P = 0.003), higher level of ALP (117.5 ± 70.2 ng/ml vs. 95.7 ± 52.9 ng/ml, p = 0.006), and lower level of albumin (39.8 ± 5.5 g/L vs. 42.0 ± 5.3 g/L, p = 0.002) (Table 3). All the factors except MaVI are related to liver cirrhosis.

To exclude the confounding effect of MaVI, we excluded patients with MaVI. The results showed that CA19-9 was still correlated with liver cirrhosis, APRI, ALT, AST, rGT, ALP, and albumin (data not shown). Furthermore, multivariate analysis showed that CA19-9 (+) and MaVI (+) were both independent risk factors for RFS.

In the current study, CA19-9 was not correlated to NLR or PLR, indicating that CA19-9 was not correlated to systemic inflammation.

Immunohistochemical staining

To determine the source of CA19-9, we examined its expression in TMA samples of HCC patients. Immunohistochemical staining of CA19-9 in both tumor tissue and non-tumor liver parenchyma specimens from HCC patients was also assessed. The results showed that none of the HCC tumor cells express CA19-9, and CA19-9 was expressed in non-tumor liver parenchyma in all patients.

Immunohistochemical staining of CA19-9 in both tumor and non-tumor liver parenchyma samples from Cohort 3 revealed that CA19-9 was expressed in 64% (87/136) of ICC tumors and 4.4% (6/136) of non-tumor liver parenchyma. Serum CA19-9 was positive (≥ 39 U/ml) in 58.1% and negative (< 39 U/ml) in 41.9% of the patients with ICC. The results that immunohistochemical staining of CA19-9 was positive only in 4.4% of ICCs indicate that serum CA19-9 mainly derives from the tumor tissue of patients with ICC, which is distinct from the dominant expression of CA19-9 in the background liver in HCC patients.

Discussion

In the current study, it is revealed that CA19-9 is associated with lower OS and RFS in both AFP (+) and AFP (-) patients. CA19-9 is associated with higher incidence of macrovascular invasion and more severe liver cirrhosis and inflammation. Importantly, immunostaining of CA19-9 showed that it is secreted by the background liver, but not by tumor cells in patients with HCC.

CA19-9 is generally recognized as a tumor marker for gastrointestinal and especially hepatobiliary cancers [21], however, CA19-9 is frequently elevated in biliary obstruction [22]. In hepatocellular carcinoma, CA19-9 elevation may stand for a subgroup of patients with partial cholangiocytic differentiation [23]. However, the significance of CA19-9 in pure HCC is still far from clear. In the current study, we excluded mixed HCC-ICC as well as cholangiocytic differentiation by two independent

pathologists, and immunohistochemical staining of CA19-9 in HCC and ICC tumor tissues as well as non-tumor liver parenchyma confirmed that CA19-9 is secreted exclusively by the background liver but not by tumor cells in patients with HCC. In contrast, CA19-9 was frequently expressed in ICC tumors (64%, n = 87/136), but rarely in the non-tumor liver parenchyma (4.4%, 6/136).

In Cohorts 1 and 2, CA19-9 ≥ 39 U/ml was associated with a high incidence of MaVI, elevated rGT, ALT, AST, PT, and APRI; and decreased albumin. Furthermore, there were no significant difference in NLR and PLR, as biomarkers of systemic inflammation, between CA19-9 (+) and CA19-9 (-) patients, indicating that CA19-9 is not associated with systemic inflammation. Previous reports have shown that elevated ALT, AST, and rGT levels are correlated to liver fibrosis, cirrhosis [24, 25], and recurrence [26, 27]. Thus, we confirmed that CA19-9 is a liver biomarker that higher serum CA19-9 level indicated more severe liver inflammation and liver cirrhosis rather than a tumor factor in HCC.

Macrovascular invasion and multiple tumor nodules were more common in CA19-9 (+) patients. These findings can be attributed to two reasons. First, chronic inflammatory diseases are the key etiological risk factors for HCC [28], and an elevated ALT/AST/APRI in patients with elevated CA19-9 indicated more severe liver cirrhosis and an inflamed liver background [29, 30], which is closely related to de novo tumor pathogenesis and multicentric recurrence [31, 32]. Second, liver inflammation may promote intravenous metastasis of HCC tumor cells and has been reported as an independent risk factor for early tumor recurrence in patients with HCC [33–35].

Persistently elevated CA19-9 levels had been reported to predict poor prognosis in HCC. Chen et al. [9] found that 34.9% (106/304) of patients with HBV- and HCV-related HCC have CA19-9 levels > 27 U/ml. Further, a preoperative CA19-9 value of > 27 U/mL was associated with poor prognosis after resection for HCC. However, the authors did not compare the clinicopathological factors related to liver inflammation and liver cirrhosis (e.g., ALT, AST, ALP, rGT, and albumin) according to a CA19-9 cutoff of ≤ 27 U/mL. Wan et al. [10] also showed that preoperative serum AFP levels of > 400 ng/ml and CA19-9 > 100 U/ml predicted survival after liver transplantation in patients with HCC. However, the study only included patients with liver transplantation and did not exclude patients with ICC or cHCC-CC. Hsu et al. [8] investigated 145 cases of HCV-related HCC and found that an elevated serum CA19-9 level of ≥ 100 U/ml is an independent predictor of poor OS. However, 59% (85/145) of the HCC patients had underlying hepatitis C virus, and only 16% had hepatitis B virus. Lu et al. [36] evaluated 750 cases of AFP-negative HCC and found that a preoperative CA19-9 level of > 32.6 U/ml predicted poor prognosis and can thus be used as a prognostic marker in AFP-negative HCC.

The current study is the only study including purely patients with HBV-related HCC. At a cut-off value of 39 U/mL, 16.1% of patients (61/380) were found to be CA19-9 positive, and CA19-9 ≥ 39 U/ml predicted worse OS and RFS in both AFP(-) and AFP (+) patients. These findings indicate that patients with CA19-9 ≥ 39 U/ml should be closely followed up considering the higher incidence of tumor recurrence and mortality rate caused by more severe liver cirrhosis and inflammation in this population. Anti-inflammatory medications may be helpful in reducing the risk of tumor recurrence.

Our study has several limitations. First, it is a single-center study of retrospective cohorts, and only the serum level and immunohistochemical expression of CA19-9 were evaluated. Second, the precise mechanism by which CA19-9 promotes macroscopic vascular invasion is still unclear and thus further studies are needed to elucidate the underlying mechanism.

Conclusions

In conclusion, CA19-9 is associated with lower OS and RFS in both AFP (+) and AFP (-) patients. Importantly, CA19-9 is secreted by the background liver, but not by tumor cells in patients with HCC. Thus, CA19-9 is not a tumor biomarker, but a biomarker for liver cirrhosis and inflammation and a risk factor for worse OS and RFS in HCC.

Abbreviations

AFP, α-fetoprotein.

ALP, Alkaline Phosphatase.

ALT, Alanine Aminotransferase.

APRI, Aspartate aminotransferase to Platelet Ratio Index.

AST, Aspartate Aminotransferase.

CA19-9, Carbohydrate Antigen 19-9.

HBeAg, Hepatitis B virus e Antigen.

HCC, Hepatocellular carcinoma

ICC, Intrahepatic cholangiocarcinoma

IHM, Intrahepatic Metastasis.

MaVI, Macroscopic Vascular Invasion.

MiVI, Microscopic Vascular Invasion.

NA, Not Applicable.

NLR, Neutrophil-to-Lymphocyte Ratio .

PLR, Platelet-to-Lymphocyte Ratio.

PT, Prothrombin Time.

rGT, Gamma-glutamyl Transpeptidase.

TB, Total Bilirubin.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from the patient for publication of their information.

Consent to publish

All authors agree to publish the article in BMC Gastroenterology.

Availability of data and materials

All data for non-commercial profit purposes can be obtained from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Authors' Contributions

ZW conceived the study concept and design and drafted the manuscript.

WYY, YB, CL, ZZW, CGT, HXY, LGH performed the acquisition of data and critical revision of the manuscript for important intellectual content. LHK, CYL, WQ, ZT, STQ and LQ assisted in clinical data and follow-up. All authors have read and approved the final manuscript.

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Wei Zhang, Ying-Ying Wang, and Bo Yang should be regarded as co-first authors.

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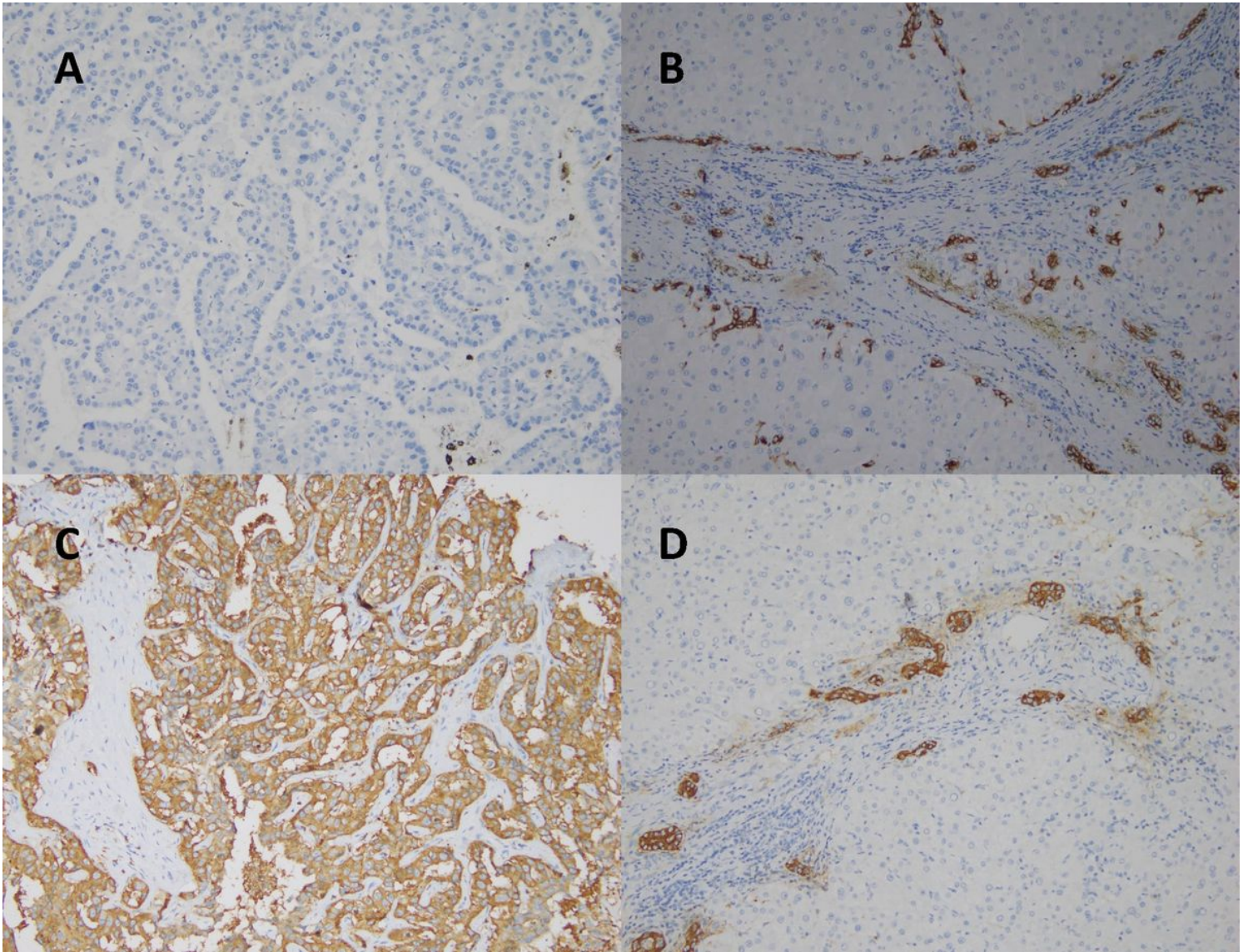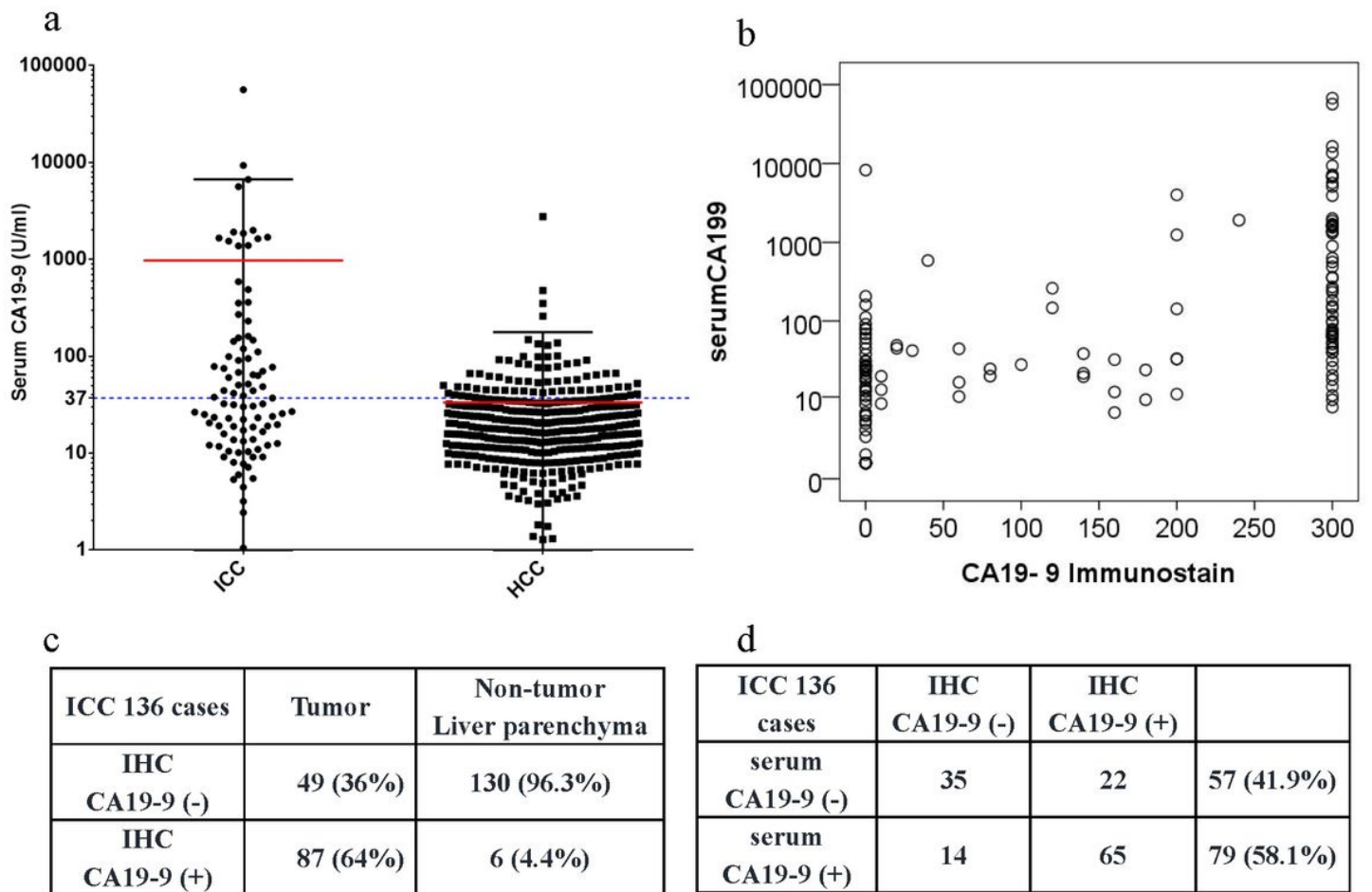
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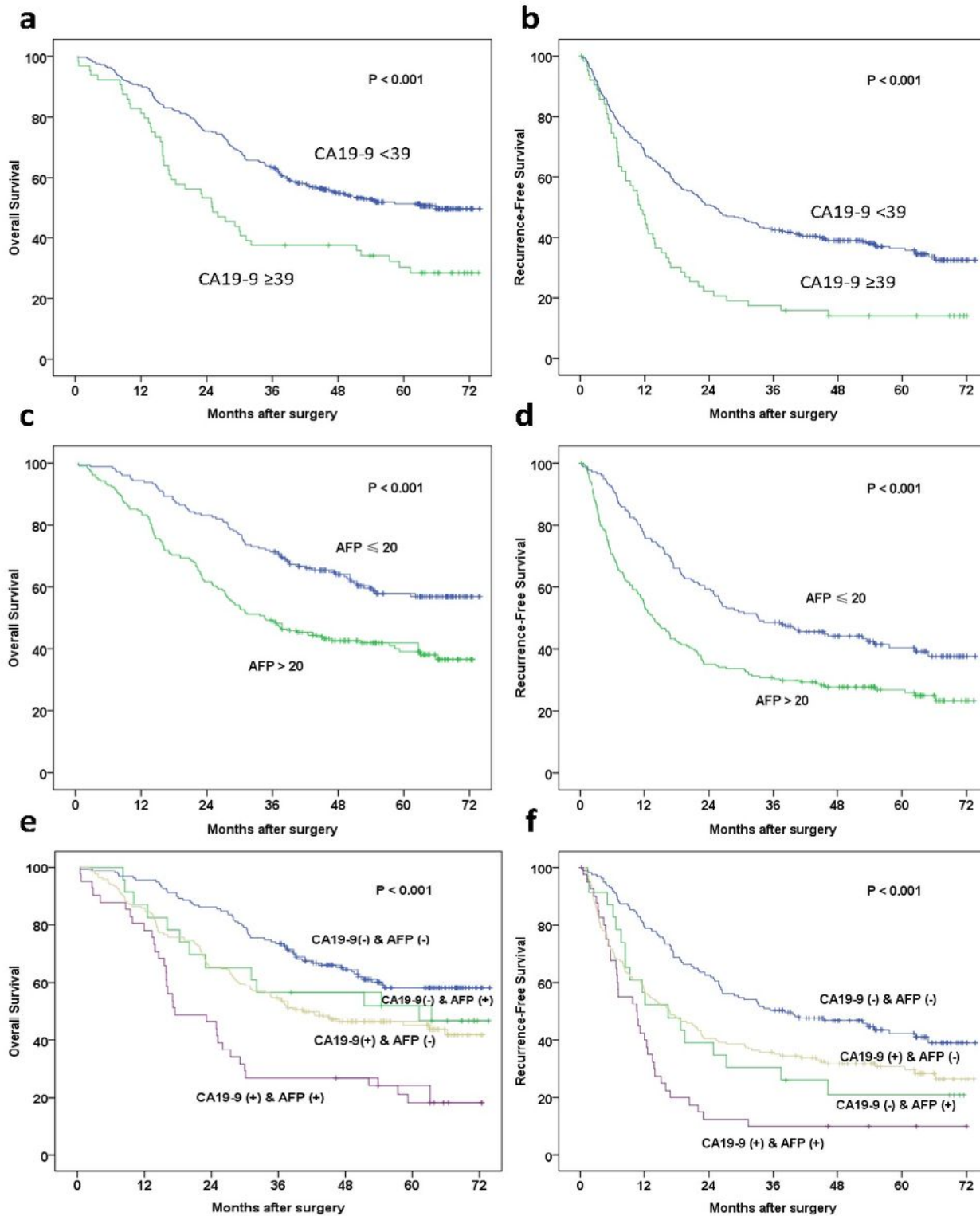
Figure 1

AFP, CA19-9 and combination to predict OS and RFS for HCC patients after curative resection.

Figure 2

Immunohistochemical staining of CA19-9 in HCC and ICC in Cohort 2 and 3. A, HCC tumor tissue was negative for CA19-9. B, HCC non-tumor liver parenchyma was positive for CA19-9 in the portal area. C, ICC tumor tissue was positive for CA19-9. D, Positive staining of CA19-9 in the portal area in non-tumor liver parenchyma of ICC.

a

b

c

ICC 136 cases	Tumor	Non-tumor Liver parenchyma
IHC CA19-9 (-)	49 (36%)	130 (96.3%)
IHC CA19-9 (+)	87 (64%)	6 (4.4%)

d

ICC 136 cases	IHC CA19-9 (-)	IHC CA19-9 (+)	
serum CA19-9 (-)	35	22	57 (41.9%)
serum CA19-9 (+)	14	65	79 (58.1%)

Figure 3

CA19-9 in HCC and ICC in Cohort 2 and 3. a, comparison of serum CA19-9 level in ICC and HCC patients. b, Serum CA19-9 correlated with IHC CA19-9 in Cohort 3 (p<0.001). c, Immunohistochemical staining of CA19-9 in ICC tumor and non-tumor liver parenchyma showed that CA19-9 is positive in 64% in tumor tissue and 4.4% in non-tumor liver parenchyma. d, comparison between serum CA19-9 and immunochemical staining of CA19-9 in ICC patients.