Diagnostic value of carbohydrate antigen CA50, carbohydrate antigen 19-9 and α-fetoprotein in biliary tract cancer: A large-scale multicenter study

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Research Article

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Abstract

Background

To date, carbohydrate antigen 19 – 9 (CA19-9) and carcinoembryonic antigen (CEA) have been widely used for the screening, diagnosis and prediction of biliary tract cancer (BTC) patients. However, few studies with large sample sizes of carbohydrate Antigen 50 (CA50) were reported in BTC patients and combined CA50, CA19-9 and α-fetoprotein (AFP) to build a clinical diagnostic model to provide a new screening and diagnosis method.

Methods

Here, we designed a cross-sectional study and analyzed data for patients with BTC, hepatocellular carcinoma (HCC), combined hepatocellular-cholangiocarcinoma (CHC), and benign biliary-liver diseases (BBD) and healthy people (HP) from two Chinese hospitals diagnosed between January 2017 and December 2022. Receiver operating characteristic (ROC) curves and decision curve analysis (DCA) were used to evaluate the diagnostic efficacy and clinical usefulness.

Results

A total of 1121 patients were included in this study (673 in the training cohort and 448 in the validation cohort): among them, 458 with BTC were included in the experimental group, and 178 with HCC, 23 with CHC, 242 with BBD, and 220 with HP were included in the control group, respectively. ROC curves by combining CA50, CA19-9 and AFP showed that, the AUC value of the diagnostic MODEL 1 was 0.885 (95% CI 0.856–0.885, specificity 93.9%, and sensitivity 74.3% in the training cohort; 0.879 (0.841–0.917, 92.8% and 75.9%) in the validation cohort. In addition, comparing iCCA and HCC (235 in the training cohort, 157 in the validation cohort), the AUC values of the diagnostic MODEL 2 was 0.893 (95% CI 0.853–0.933, specificity 96%, and sensitivity 68.6%) in the training cohort; 0.872 (95% CI 0.818–0.927, 94.2%, and 64.6%) in the validation cohort.

Conclusion

The model combining CA50, CA19-9, and AFP not only has good diagnostic value for BTC, but also has good diagnostic value for distinguishing iCCA and HCC.

Background

Biliary tract cancer (BTC) comprises a group of malignancies originating in the epithelium of the biliary tract, and is a relatively rare cancer worldwide. However, it is prevalent in Asia [1, 2]. A lack of robust screening measures, no specific clinical symptoms in the early stage, and late diagnosis (unresectable to metastatic) contribute to poor overall survival in BTCs [3]. Therefore, it is very important for early accurate screening and diagnosis of BTCs.

BTCs are classified into five types, including intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), distal cholangiocarcinoma (dCCA), vater ampulla carcinoma (VPC), and carcinoma of the gallbladder (GBC), based on anatomical location to help develop a therapeutic treatment plan clinically [4]. Among them, a definitive diagnosis of iCCA requires liver biopsy analysis, because it is difficult to distinguish iCCA from hepatocellular carcinoma (HCC) or combined hepatocellular-cholangiocarcinoma (CHC) by imaging alone [5]. Therefore, to distinguish between iCCA and HCC and CHC patients before treatment, the development of efficient tumor biomarkers remains pivotal.

Traditionally, there are several BTC biomarkers that have been widely used clinically to screen for the disease early, diagnose the disease, set prognoses, and determine the efficacy of treatments, such as carbohydrate antigen 19 – 9 (CA19-9) and carcinoembryonic antigen (CEA) [6–10]. The sensitivity of CA19-9 and CEA is high, but their specificity is poor. Other investigators have questioned the utility of these tumor markers in predicting the development of cholangiocarcinoma or other pancreaticobiliary tumors due to a high false positive rate [11–13]. Thus, exploring and discovering novel specific effective biomarkers will facilitate the identification of BTCs and other biliary tract malignancies.

Previously, carbohydrate antigen 50 (CA50) was reported to be a ganglioside glycoprotein and played an important clinical role in the screening and diagnosis of gastrointestinal malignancies, particularly pancreatic and colon cancer [14–17]. Despite extensive research on the screening and diagnostic value of CA50, its powerful evidence and data in BTC or CHC patients have rarely been explored. Only a few studies with small sample sizes have confirmed the high expression of CA50 in CCA [18, 19]. However, to date, the exact clinical value of CA50 in BTCs remains unclear. Whether CA50 was more effective in combination with other tumor markers was also unclear.

Thus, we designed a large-scale multicenter study to explore the screening and diagnostic values of CA50, CA19-9, CEA and α-fetoprotein (AFP) in BTC patients and to address the following questions. First, we clarified the relationship among serum CA50, CA19-9, AFP, and CEA levels and BTC patients, as well as its optimal threshold, sensitivity, specificity, accuracy and so on. Second, we found that CA50 was significantly correlated with CA19-9 in BTC patients. But CA50 was a neglected tumor marker with high specificity. Furthermore, we combined CA50, CA19-9 and AFP to build a clinical diagnostic model to help differentiate iCCA patients from HCC patients before treatment.

Methods

Study Design

The clinical data of 4230 cancer patients from two centers of the Liver Cancer Clin-Bio Databank of Anhui Hepatobiliary Surgery Union (LCCBD_AHSU) and 462 non-tumors from the Physical Examination Center of the First Affiliated Hospital of the University of Science and Technology in China were collected from January 2017 to December 2022. LCCBD_AHSU was collected from a prospectively maintained database and reviewed retrospectively. Among these, 1121 patients were included, and the remaining 3571 patients who met the exclusion criteria were excluded. Then, 458 patients with BTC, 178 patients with HCC, 220 healthy people (HP), 23 patients with CHC, and 242 patients with benign biliary disease (BBD) were collected. All patients were randomly divided into a training cohort and a validation cohort according to 6:4 (673: 448). In addition, based on the tumor location, BTCs were divided into five categories, including 214 cases of iCCA, 62 cases of pCCA, 66 cases of dCCA, 78 cases of GBC and 38 cases of VPC. To differentiate iCCA patients from HCC patients before treatment, 392 patients were selected and were also randomly divided at a 6:4 ratio (235: 157). The study protocol was approved by the Ethics Committee (ID: 2023-KY-24). Due to the retrospective nature of the study, the demand for obtaining a written informed consent form that the patient signed was waived (Fig. 1). This trail was registered in the Chinese Clinical Trail Registry (ChiCTR), ChiCTR2300069682.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) cancer patients diagnosed with BTC, HCC, or CHC by pathological diagnosis; (2) patients receiving AFP, CA19-9, CEA, or CA50 examinations; and (3) patients receiving imaging examinations.

The exclusion criteria were as follows: (1) lack of the serum tumor biomarkers or imaging train data that were analyzed in this study; and (2) missing the other clinical data that were analyzed in the study.

The enrolled patients were required to meet all three inclusion criteria, and patients meeting any of the exclusion criteria were excluded.

Tumor marker analysis

The serum tumor marker levels were examined by an automatic CL-6000i chemiluminescence immunoassay (CLIA) analyzer from Mindray Co, Ltd. (Mindray Industries Biomedical Engineering), Shenzhen, China.

Due to the limitations of laboratory reports, the serological results of some patients were reported as follows: CA50 > 500 U/ml, CA19-9 > 1000 U/ml, AFP > 1200 ng/mL, and CEA > 1000 ng/ml. In addition, the results of the above patients were statistically analyzed using the maximum critical value of their reports. The level of tumor markers was normally displayed in the Table. Due to the large partial maximum value, logarithmic transformation was taken in the Figure considering the aesthetics of the picture.

Statistical Analysis

The statistical analyses were performed using IBM-SPSS statistics (Version 26) and R language (Version 4.2.2). Normally distributed data are described as the mean ± standard deviation (SD). Skewed distribution data are described as medians (min, max). Normally

distributed data combined with variance homogeneity were compared between multiple groups by *ANOVA*. Skewed distribution data or heterogeneity of variance were compared between multiple groups by the *Kruskal–Wallis H* train. The correlation analysis was compared between two groups by Spearman nonparametric linear analysis. The level of tumor markers was normally displayed in the Table. Due to the large partial maximum value, logarithmic transformation was taken in the Figure considering the aesthetics of the picture. Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR), negative LR and respective areas under the curves (AUCs) with 95% CIs. Decision curve analysis (DCA) and a nomogram were used to evaluate the clinical usefulness of the model. The diagnostic model was constructed by logistic regression and included CA50, CA19-9 and AFP. **MODEL 1: logit= -1.83862 + 0.02204*CA50 + 0.01214*CA199 -0.00245*AFP. MODEL 2: logit= -0.75580 + 0.00766*CA50 + 0.01554*CA19.9 -0.00999*AFP.** $P < 0.05$ was considered statistically significant.

Results

Levels of tumor biomarkers in the BTC, HCC, CHC, BBD, and HP groups

To investigate the expression levels of different tumor markers in different types of populations, the preoperative expression levels of CA50, CA19-9, AFP, and CEA in BTC patients were measured and compared with those of non-BTC controls (**Figure 2**). The median levels of serum CA50 in the BTC, HCC, CHC, BBD and HP groups were 61.5 U/ml, 13.6 U/ml, 7.7 U/ml, 5.6 U/ml and 5.9 U/ml, respectively. The median levels of serum CA19-9 in the BTC, HCC, CHC, BBD and HP groups were 120.3 U/ml, 11.2 U/ml, 17.3 U/ml, 7.7 U/ml and 9.7 U/ml, respectively. The median levels of serum AFP in the above groups were 3.1 ng/ml, 15.1 ng/ml, 14.0 ng/ml, 2.8 ng/ml and 2.3 ng/ml, respectively. The median levels of serum CEA in the above groups were 3.3 ng/ml, 2.5 ng/ml, 2.4 ng/ml, 1.7 ng/ml and 1.6 ng/ml, respectively (**Table 1, Figure 2**). The levels of serum CA50, CA19-9, and CEA in the BTC patients were higher than those in the HCC, CHC, BBD, and HP patients, and the difference was significant (P <0.001) (**Table 1**).

In addition, correlation analysis of CA50 with other tumor markers is presented in **Figure 3**. The serum CA50 was significantly correlated with serum CA19-9. The R-values in BTC, HCC, CHC, BBD, and HP were 0.85, 0.62, 0.76, 0.89, and 0.95, respectively (**Figure 3A**). However, the serum CA50 was not significantly correlated with serum AFP or CEA, and the R-values were all small in the above patients (**Figure 3B and Figure 3C**).

Therefore, the levels of serum CA50, CA19-9, and CEA in the BTC patients were higher than those in HCC, CHC, BBD, and HP patients. In addition, the levels of serum CA50 and CA19-9 were significantly correlated in BTC, CHC, BBD, and HP patients.

Diagnostic values of serum tumor markers and the model in BTC patients

A total of 1121 persons were randomly divided into the train cohort and the validation cohort according to 6: 4 (673: 448). There were not significant statistical differences in age, sex, different levels of serum tumor markers, maximum tumor, and study population between the train cohort and the validation cohort **(Table 2)**.

Comparing BTC and non-BTC, the best cut-off value of the single serum CA50 level was 24.875 U/ml (train cohort: AUC 0.841, 95% CI 0.808-0.875, specificity 92.2%, sensitivity 68.0%; validation cohort: AUC 0.833, 95% CI 0.789-0.876, specificity 91.7%, sensitivity 65.6%)**.** The best cut-off value of CA19-9 was 31.680 U/ml (train cohort: AUC 0.851, 95% CI 0.818-0.885, specificity 93.2%, sensitivity 71.9%; validation cohort: AUC 0.859, 95% CI 0.819-0.899, specificity 92.5%, sensitivity 69.8%). The best cut-off value of AFP was 3.105 ng/ml (train cohort: AUC 0.476, 95% CI 0.430-0.521, specificity 52.6%, sensitivity 51.9%; validation cohort: AUC 0.498, 95% CI 0.442-0.553, specificity 57.3%, sensitivity 46.4%). And the best cut-off value of CEA was 3.230 ng/ml (train cohort: AUC 0.715, 95% CI 0.673-0.757, specificity 78.6%, sensitivity 53.1%; validation cohort: AUC 0.704, 95% CI 0.653-0.756, specificity 80.4%, sensitivity 48.8%) **(Table 3, Figure 4)**. The AUC of serum CA19-9 and CA50 were higher than that of AFP and CEA, and serum CA50 and CA19-9 had a better specificity and positive LR. The accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR) and negative LR of the different tumor markers are also shown in **Table 3**. In addition, we built a clinical diagnostic model by combining CA50, CA19-9 and AFP, and the AUCs of the MODEL 1 was 0.885 (train cohort: 95% CI 0.856-0.915, specificity 93.9%, sensitivity 74.3%) and 0.879 (validation cohort: 95% CI 0.841-0.917, specificity 92.8%, sensitivity 75.9%), which was higher than that of the above serum tumor markers alone **(Figure 4A, Figure 4B)**. The results of DCAs also indicated that the MODEL 1 could add more benefit than the “treat none” and “treat all” **(Figure 4C, Figure 4D)**.

In the MODEL 1, we used the following formula: **logit= -1.83862 +0.02204*CA50 +0.01214*CA199 -0.00245*AFP.** The nomogram of the MODEL 1 was shown in **Figure 5**.

Therefore, CA50 is a potential tumor marker for the screening and diagnosis of BTC patients. Distinguish BTC and non-BTCs patients, serum CA50 and CA19-9 had a better specificity and positive LR than those of AFP and CEA. The AUCs of the MODEL 1 by combining CA50, CA19-9 and AFP were higher than those of the single serum tumor markers.

Diagnostic values of different tumor markers and the model in iCCA patients

A total of 392 persons were randomly divided into the train cohort and the validation cohort according to 6: 4 (235: 157). There were not significant statistical differences in age, sex, different levels of serum tumor markers, maximum tumor, and study population between the train cohort and the validation cohort **(Table 4).**

Distinguishing iCCA and HCC, the best cut-off value of serum CA50 was 52.995 U/ml (train cohort: AUC 0.752, 95% CI 0.688-0.816, specificity 93.5%, sensitivity 58.6%; validation cohort: AUC 0.698, 95% CI 0.612-0.783, specificity 94.4%, sensitivity 48.8%). And the best cut-off value of serum CA19-9 was 52.700 U/ml (train cohort: AUC 0.834, 95% CI 0.781-9.887, specificity 90.7%, sensitivity 68.0%; validation cohort: AUC 0.781, 95% CI 0.708-0.854, specificity 91.6%, sensitivity 58.8%). The best threshold of AFP was 6.750 ng/ml (train cohort: AUC 0.761, 95% CI 0.697-0.825, specificity 58.4%, sensitivity 85.6%; validation cohort: AUC 0.702, 95% CI 0.614-0.790, specificity 59.4%, sensitivity 84.2%). And the best threshold of CEA was 3.325 ng/ml (train cohort: AUC 0.672, 95% CI 0.595-0.748, specificity 68.0%, sensitivity 61.1%; validation cohort: AUC 0.550, 95% CI 0.450-0.651, specificity 53.9%, sensitivity 50.6%) **(Table 5, Figure 6)**. The accuracy, PPV, NPV, positive LR and negative LR of the different tumor markers are also shown in **Table 5**. In addition, we also built a clinical diagnostic model by combining CA50, CA19-9 and AFP, and the AUCs of the MODEL 2 was 0.893 (train cohort: 95% CI 0.853-0.933, specificity 96.0%, sensitivity 68.6%) and 0.872 (validation cohort: 95% CI 0.818-0.927, specificity 94.2%, sensitivity 64.6%), which was higher than that of the above tumor marker alone **(Figure 6A, Figure 6B)**. The results of DCAs also indicated that the MODEL 2 could add more benefit than the “treat none” and “treat all” **(Figure 6C, Figure 6D).** In the MODEL 2, we used the following formula: **logit= -0.75580 +0.00766*CA50 +0.01554*CA19.9 -0.00999*AFP.** The nomogram of the MODEL was shown in **Figure 7**.

Therefore, compared to CA19-9, AFP, and CEA, serum CA50 had a better specificity and positive LR in iCCA patients. The AUCs of the MODEL 2 by combining CA50, CA19-9 and AFP were higher than those of the single serum tumor markers.

Clarification of the relationship between different tumor marker levels and BTC location

BTCs were classified into five types, including iCCA, pCCA, dCCA, GBC, and VPC, based on their anatomic location. A total of 458 BTC patients were classified, 214 with iCCA, 62 with pCCA, 66 with dCCA, 78 with GBC, 38 with VPC. The overall distribution of the different tumor markers in different locations of BTC was shown in **Supplementary Figure 1**. The median levels of serum CA50 in the iCCA, pCCA, dCCA, GBC and VPC patients were respectively 66.5 U/ml, 95.3 U/ml, 61.3 U/ml, 30.9 U/ml and 77.7 U/ml, respectively. The median levels of serum CA19-9 in the iCCA, pCCA, dCCA, GBC and VPC patients were respectively 134.4 U/ml, 231.9 U/ml, 62.1 U/ml, 62.5 U/ml, and 104.8 U/ml respectively (**Supplementary Table 1**). The median levels of serum AFP in the iCCA, pCCA, dCCA, GBC and VPC patients were respectively 3.2 U/ml, 3.4 U/ml, 2.9 U/ml, 2.4 U/ml and 2.5 U/ml. And the median levels of serum CEA in the above locations were 3.5 ng/ml, 3.7 ng/ml, 2.9 ng/ml, 2.3 ng/ml and 3.5 ng/ml, respectively. The serum CA19-9, AFP, and CEA values were significantly different at different locations of BTCs (P =0.020, P =0.026, P =0.001). (**Supplementary Figure 1, Supplementary Table 1**).

Therefore, the levels of serum AFP, CA19-9 and CEA were correlated with the location of BTCs. The serum CA50 and CA19-9 levels were expressed at higher levels in pCCA than in iCCA, dCCA, GBC and VPC.

Clarification of the relationship between different tumor marker levels and the degree of jaundice

Based on the total bilirubin levels (TBIL), patients with BTC were divided into five groups: no jaundice group (TBIL <17.1 μmol/L), recessive jaundice group (TBIL: 17.1-34.2 μmol/L), mild jaundice group (TBIL: 34.3-171.0 μmol/L), moderate jaundice group (TBIL: 172.0-342.0 μmol/L) and severe jaundice group (TBIL >342.0 μmol/L) (**Supplementary Figure 2**). The median serum CA50 levels in the above five groups were 28.5 U/ml, 65.3 U/ml, 80.7 U/ml, 132.0 U/ml, 312.8 U/ml, and the median serum CA19-9 levels in the above five groups were 53.6 U/ml, 121.4 U/ml, 143.6 U/ml, 173.8 U/ml, 522.9 U/ml. The serum levels of both CA50 and CA19-9 increased with the severity of jaundice, and the difference was statistically significant (P <0.001, P =0.010). There was no significant difference

between the serum CEA and AFP levels and the degree of jaundice in different groups (P =0.721, P =0.583) (**Supplementary Table 2, Supplementary Figure 2**).

Therefore, the serum CA50 and CA19-9 levels increased with the degree of jaundice.

Clarification of the relationship between different tumor marker levels and the degree of pathological pattern

Based on the pathological examination results, patients with BTC were divided into four groups: poorly differentiated adenocarcinoma, moderately differentiated adenocarcinoma, High differentiated adenocarcinoma, and other types (**Supplementary Figure 3**). Other types including squamous cell carcinoma, adeno-squamous carcinoma, neuroendocrine carcinoma and so on. The median serum CA50 levels in the above four groups were 66.6 U/ml, 63.8 U/ml, 35.2 U/ml, 46.3 U/ml, and the median serum CA19-9 levels in the above four groups were 134.4 U/ml, 102.9 U/ml, 60.4 U/ml, 110.1 U/ml. The serum levels of CA50 and CA19-9 were highest in the poorly differentiated adenocarcinoma. The serum levels of CA19-9 increased with the severity of jaundice (P =0.048). There was no significant difference between the serum CA50, CEA, and AFP levels and the degree of jaundice in different groups (P =0.358, P =0.795, P =0.294) (**Supplementary Table 3, Supplementary Figure 3**).

Therefore, the serum CA19-9 levels were obviously higher in the poorly differentiated adenocarcinoma than in the high differentiated adenocarcinoma in BTCs.

Discussion

Traditionally, many guidelines and studies have recommended CA19-9 and CEA as screening and diagnostic markers for BTC or iCCA patients. Few studies have reported the potential diagnostic value of CA50 in these patients [18.19]. Some studies have proven that altered glycosylation occurs in BTC, and the abnormal expression of glycans plays a significant role in the progression of BTC, resulting in poor survival outcomes for patients [20–24]. Therefore, it was discovered that BTC-associated glycans might be the potential and predictive biomarkers for diagnosis and prognosis [23, 25, 26]. In fact, both CA50 and CA19-9 are the sialyl-associated glycan antigens. As our study has shown, CA50 and CA19-9 were highly correlated with each other in BTC, CHC, BBD and HP patients. However, CA50 is rarely used clinically and few guidelines recommend training in patients with BTC. Moreover, few studies have explored the diagnostic value of CA50 in combination with other tumor markers.

As previous studies reported by Haglund et al. [19], Luang et al. [18], and Watanabe et al [27] have shown, CA50 levels could be secreted from CCA tissues and were abnormally elevated in CCA tissues [18, 19, 27]. However, the sample sizes of the above studies were small, and the patient data analyzed did not include GBC, VPC, CHC and so on. To date, the exact clinical value of CA50 in BTCs remains inaccuracy. Our multicenter large-sample size study has detailed the relationship between serum CA50 levels and BTC patients, including the optimal threshold, sensitivity, specificity, accuracy, PPV, NPV, positive LR, negative LR and AUCs with 95% CI. In this study, the levels of serum CA50, CA19-9, and CEA in BTC patients were higher than those in non-BTC patients, and the difference was significant. In addition, the ROC results indicated that CA50 was a potential tumor marker for the screening and diagnosis of BTC patients. Compared to AFP, and CEA, serum CA50 and CA19-9 had a better specificity and positive LR in BTCs. Moreover, the clinical diagnosis model combining CA50, CA19-9 and AFP also showed better diagnostic performance than the single tumor marker. But the clinical diagnosis model showed brilliant specificity and moderate sensitivity. As a result, we considered that the diagnostic model might be more suitable for diagnosis and whether it could solve an important clinical problem, that is the preoperative diagnosis of iCCA and HCC.

As we known, BTCs are usually classified clinically according to anatomical location. Patients with different classifications have significantly different survival prognoses and treatment strategies. In fact, it is difficult to distinguish iCCA from HCC and CHC only by imaging before obtaining pathological results [5]. However, the choice of treatment plan depends greatly on a correct preoperative diagnosis. As a small sample size study concluded, CA50 could be a diagnostic marker candidate for iCCA [18]. However, the control group of the above study only had BBD, HP and a few other gastrointestinal cancers and lacked a large number of HCC and CHC patients, who were difficult to distinguish before treatment. In our study, the specificity and positive LR of serum CA50 were also higher than those of CA19-9, and CEA in iCCA, but the sensitivity of serum CA50 was lower than others. Consider the clinical feature of CA50, we built a clinical diagnostic model again by combining CA50, CA19-9 and AFP to help differentiate between HCC and iCCA patients. The model did not include CEA because it has a poor AUC value according to our large sample size results. The AUCs of the

MODEL 2 were 0.885 in the train cohort and 0.879 in the validation cohort, which were higher than those of the single serum CA50, CA19-9, AFP and CEA. The results of DCAs also indicated that the MODEL 2 could add more benefit than the “treat none” and “treat all”. Therefore, the diagnostic model has high diagnostic value and can assist in distinguishing iCCA and HCC without relying on imaging.

In the clinic, the degree and type of pathological differentiation, tumor location and degree of jaundice in BTC patients are all important. However, few studies have explored the correlation between different tumor markers and these clinical information of BTCs. In our study, we found that the expression levels of CA50 and CA19-9 were higher in pCCA than in VPC, iCCA, dCCA, and GBC. We hypothesized that this difference in expression might be related to the higher incidence of obstruction of the biliary tract in pCCA. As a previous study reported by Luang S et al, in iCCA patients, patients with jaundice expressed higher levels of CA50 than those without jaundice [18]. Therefore, we compared the levels of tumor markers with different degrees of jaundice who were divided into five groups: no jaundice, recessive jaundice, mild jaundice, moderate jaundice and severe jaundice groups. The serum levels of both CA50 and CA19-9 increased with the severity of jaundice, and the difference was statistically significant. Besides, the serum CA19-9 levels were obviously higher in the poorly differentiated adenocarcinoma than in the high differentiated adenocarcinoma in BTCs. This phenomenon may suggest that the higher the level of CA19-9, the worse the prognosis of patients. Therefore, both CA50 and CA19-9 are not only sialyl-associated glycan antigens but also have similar clinical features. CA50 seems to be a neglected tumor biomarker that has great clinical diagnostic value in BTCs.

However, there were still some limitations in our study. First, the study included only Asian populations, and the results may have some differences in other populations. Second, our BBD patients included few patients with primary biliary sclerosis due to retrospective limitations. Third, we did not investigate the relationship between serum markers and tumor recurrence or patient survival, which will be further explored through prospective studies.

Conclusion

To our knowledge, this is the first large-scale multicenter study to add neglected CA50 to the clinical diagnostic model of BTCs, which has good diagnostic value in distinguishing iCCA from HCC by combining CA50, CA19-9, and AFP.

Abbreviations

BTC, biliary tract cancer; CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; dCCA, distal cholangiocarcinoma; VPC, vater ampulla carcinoma; GBC, carcinoma of gallbladder; HCC, hepatocellular carcinoma; CHC, combined hepatocellular-cholangiocarcinoma; BBD, benign biliary-liver diseases; HP, healthy people; ROC, receiver operating characteristic curve; AUC, area under curve; DCA, decision curve analysis; CA50, carbohydrate Antigen 50; CA19-9, carbohydrate antigen 19 – 9; CEA, carcinoembryonic antigen; AFP, α-fetoprotein; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; TBIL, total bilirubin level

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China (ID: 2023-KY-24). This trail was registered in the Chinese Clinical Trail Registry (ChiCTR), ChiCTR2300069682.

Consent for publication

Due to the retrospective nature of the study, the demand for obtaining a written informed consent form that the patient signed was waived.

Availability of data and materials

The data generated in this study are available upon request from the corresponding author.

Competing interests

No conflict of interest exists in the submission of this manuscript.

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Authors' contributions

Guarantor of the article: Ji-Zhou Wang; Study design: Yong-Shuai Wang; Manuscript draft and analysis: Yong-Shuai Wang; Analysis and data visualization: Wei Wang (Department of Oncology); Data acquisition: Zheng Lu, Fei-Yu Qi, Wei Cai, Sai Zhang, Shen-Yu Zhang, Wang Wei (Department of Pathology), Hua-Chuan Song, Huan-Zhang Yao, Fan-Zheng Meng, Tao Mei and Rui-Peng Song; Critical revisions: Lian-Xin Liu.

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Tables

Table 1. Levels of tumor markers in the BTC, HCC, CHC, BBD, and HP groups.

	BTC (n =458	HCC (n =178)	CHC (n =23)	BBD (n =242)	HP (n =220)	*P*
Median CA50 (min, mix), U/ml	61.5 (0.4-2412.2)	13.6 (0.5-163.7)	7.7 (0.5-939.2)	5.6 (1.0-104.2)	5.9 (1.0-21.1)	<0.001
Median CA19-9 (min, mix), U/ml	120.3 (0.6-5355.0)	11.2 (0.6-472.8)	17.3 (0.6-1888.5)	7.7 (1.0-133.5)	9.7 (1.0-41.1)	<0.001
Median AFP (min, mix), ng/mL	3.1 (0.8-2933.0)	15.1 (0.8-5400.0)	14.0 (2.1-1210.0)	2.8 (0.8-120.8)	2.3 (0.5-18.6)	<0.001
Median CEA (min, mix), ng/mL	3.3 (0.4-1160.3)	2.5 (0.5-268.1)	2.4 (1.5-16.5)	1.7 (0.4-9.3)	1.6 (0.5-9.5)	<0.001

Note: biliary tract cancer (BTC); hepatocellular carcinoma (HCC); combined hepatocellular-cholangiocarcinoma (CHC); benign biliary-liver diseases (BBD); healthy people (HP); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); α-fetoprotein (AFP); carcinoembryonic antigen (CEA);

Table 2. Characteristic of study population in both train cohort and validation cohort (BTC *vs.* HCC+ CHC +BBD + HP) .

Characteristic	Train cohort (n=673)	Validation cohort (n=448)	*P*
Mean Age (±SD), years	56.4 (13.5)	56.6 (14.3)	0.766
Sex, n (%)			0.922
Male	361 (53.7%)	242 (54.0%)	
Female	311 (46.3%)	206 (46.0%)	
Median CA50 (min, mix), U/ml	11.2 (0.4-1050.7)	9.8 (0.5-2412.2)	0.233
Median CA19-9 (min, mix), U/ml	15.8 (0.6-5355.0)	15.0 (0.6-4421.0)	0.232
Median AFP (min, mix), ng/mL	3.1 (0.7-4277.9)	2.9 (0.5-5400.0)	0.117
Median CEA (min, mix), ng/mL	2.3 (0.4-543.0)	2.2 (0.5-1160.3)	0.231
Median Maximum tumor diameter (min, mix), mm	37.0 (1.0-170.0)	40.0 (4.0-173.0)	0.553
Study Population, n (%)			1.000
BTC	275 (40.9%)	183 (40.8%)	
HCC	107 (15.9%)	71 (15.8%)	
CHC	14 (2.1%)	9 (2.0%)	
BBD	145 (21.5%)	97 (21.7%)	
HP	132 (19.6%)	88 (19.6%)	

Note: biliary tract cancer (BTC); hepatocellular carcinoma (HCC); combined hepatocellular-cholangiocarcinoma (CHC); benign biliary-liver diseases (BBD); healthy people (HP); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); α-fetoprotein (AFP); carcinoembryonic antigen (CEA);

Table 3. ROC results of different serum tumor markers in the diagnosis of BTC *vs.* HCC+ CHC +BBD + HP.

	N	AUC (95% CI)	Cut-off Value	Specificity (%)	Sensitivity (%)	Accuracy	PPV (%)	NPV (%)	Positive LR	Negative LR
Train Cohort (BTC *vs.* HCC+ CHC +BBD + HP)										
CA50	673	0.841 (0.808-0.875)	24.875	92.2	68.0	0.823	85.8	80.7	8.730	0.347
CA19-9	672	0.851 (0.818-0.885)	31.680	93.2	71.9	0.845	88.0	82.8	10.598	0.302
AFP	631	0.476 (0.430-0.521)	3.105	52.6	51.9	0.526	40.3	63.9	1.093	0.916
CEA	602	0.715 (0.673-0.757)	3.230	78.6	53.1	0.683	62.6	71.2	2.475	0.597
MODEL 1	631	0.885 (0.856-0.915)	-0.944	93.9	74.3	0.864	88.2	85.5	12.070	0.274
Validation Cohort (BTC *vs.* HCC+ CHC +BBD + HP)										
CA50	448	0.833 (0.789-0.876)	24.875	91.7	65.6	0.810	0.845	0.794	7.899	0.375
CA19-9	447	0.859 (0.819-0.899)	31.680	92.5	69.8	0.832	0.864	0.817	9.246	0.327
AFP	428	0.498 (0.442-0.553)	3.105	57.3	46.4	0.530	0.407	0.628	1.085	0.937
CEA	409	0.704 (0.653-0.756)	3.230	80.4	48.8	0.677	0.625	0.701	2.490	0.637
MODEL 1	428	0.879 (0.841-0.917)	-1.129	92.8	75.9	0.862	0.869	0.859	10.467	0.260

Note: receiver operating characteristic curve (ROC); area under the curve (AUC); positive predictive value (PPV); negative predictive value (NPV); likelihood ratio (LR); biliary tract cancer (BTC); hepatocellular carcinoma (HCC); combined hepatocellular-cholangiocarcinoma (CHC); benign biliary-liver diseases (BBD); healthy people (HP); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); α-fetoprotein (AFP); carcinoembryonic antigen (CEA); MODEL (CA50 + CA19-9 + AFP);

Table 4. Characteristic of study population in both train cohort and validation cohort (iCCA *vs.* HCC).

Characteristic	Train cohort (n=235)	Validation cohort (n=157)	*P*
Mean Age (±SD), years	61.7 (10.5)	61.0 (10.4)	0.538
Sex, n (%)			0.719
Male	150 (63.8%)	103 (65.6%)	
Female	85 (36.2%)	54 (34.4%)	
Median CA50 (min, mix), U/ml	22.3 (0.4-2412.2)	19.6 (0.5-500.0)	0.183
Median CA19-9 (min, mix), U/ml	30.3 (0.6-5355.0)	25.4 (0.6-5353.0)	0.187
Median AFP (min, mix), ng/mL	3.9 (0.8-5400.0)	4.3 (0.8-3291.0)	0.705
Median CEA (min, mix), ng/mL	3.3 (0.5-1000.0)	3.3 (0.6-1160.3)	0.791
Median Maximum tumor diameter (min, mix), mm	230 (53.5)	154 (53.8)	0.932
Study Population, n (%)			0.952
iCCA	128 (54.5%)	86 (54.8%)	
HCC	107 (45.5%)	71 (45.2%)	

Note: intrahepatic cholangiocarcinoma (iCCA); hepatocellular carcinoma (HCC); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); carcinoembryonic antigen (CEA); α-fetoprotein (AFP);

Table 5. ROC results of different serum tumor markers in the diagnosis of iCCA *vs.* HCC.

	N	AUC (95% CI)	Cut-off Value	Specificity (%)	Sensitivity (%)	Accuracy	PPV (%)	NPV (%)	Positive LR	Negative LR
Train Cohort (iCCA *vs.* HCC)										
CA50	235	0.752 (0.688-0.816)	52.995	93.5	58.6	0.745	0.915	0.654	8.957	0.443
CA19-9	234	0.834 (0.781-0.887)	52.700	90.7	68.0	0.783	0.897	0.703	7.273	0.353
AFP	221	0.761 (0.697-0.825)	6.750	58.4	85.6	0.731	0.706	0.776	2.058	0.247
CEA	196	0.672 (0.595-0.748)	3.325	68.0	61.1	0.639	0.734	0.546	1.905	0.573
MODEL 2	221	0.893 (0.853-0.933)	0.389	96.0	68.6	0.813	0.953	0.724	17.333	0.327
Validation Cohort (iCCA *vs.* HCC)										
CA50	157	0.698 (0.612-0.783)	52.995	94.4	48.8	0.694	0.913	0.604	8.669	0.542
CA19-9	157	0.781 (0.708-0.854)	52.700	91.6	58.8	0.737	0.893	0.650	6.961	0.450
AFP	149	0.702 (0.614-0.790)	6.750	59.4	84.2	0.713	0.759	0.759	2.074	0.267
CEA	128	0.550 (0.450-0.651)	3.325	53.9	50.6	0.519	0.631	0.412	1.097	0.917
MODEL 2	149	0.872 (0.818-0.927)	0.240	94.2	64.6	0.782	0.930	0.692	11.149	0.375

Note: intrahepatic cholangiocarcinoma (iCCA); hepatocellular carcinoma (HCC); receiver operating characteristic curve (ROC); area under the curve (AUC); positive predictive value (PPV); negative predictive value (NPV); likelihood ratio (LR); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); carcinoembryonic antigen (CEA); α-fetoprotein (AFP); MODEL (CA50 + CA19-9 + AFP);

Figures

Figure 1

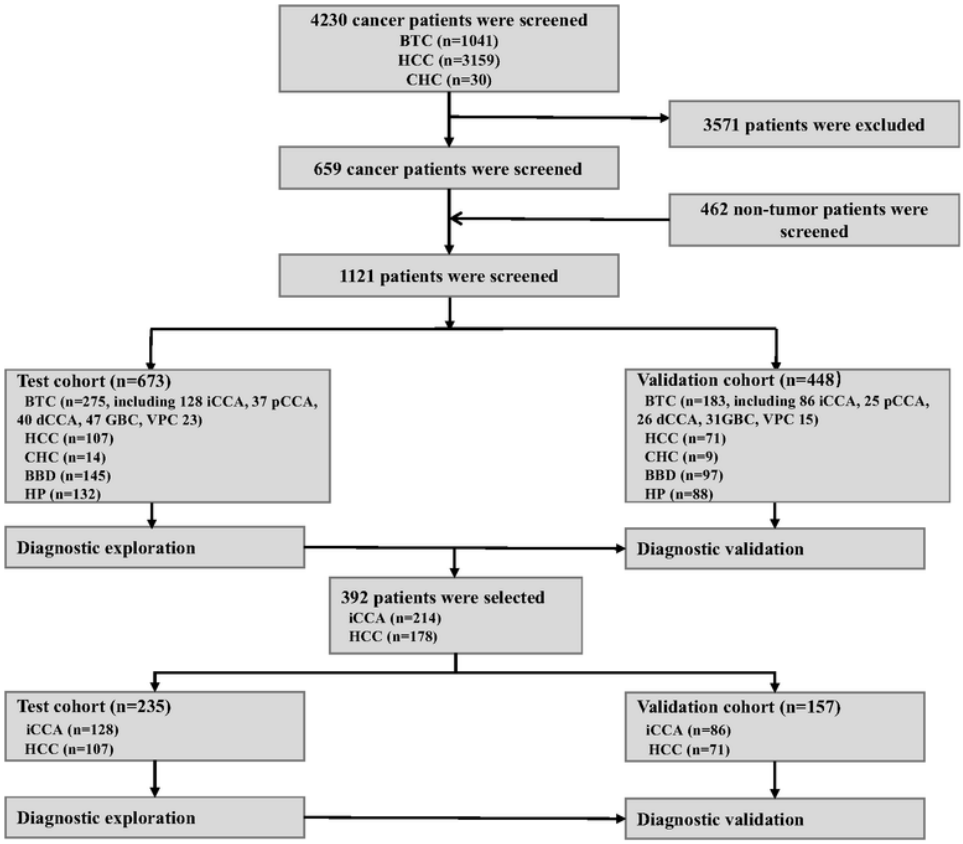

Figure 1

Flow chart of the study.

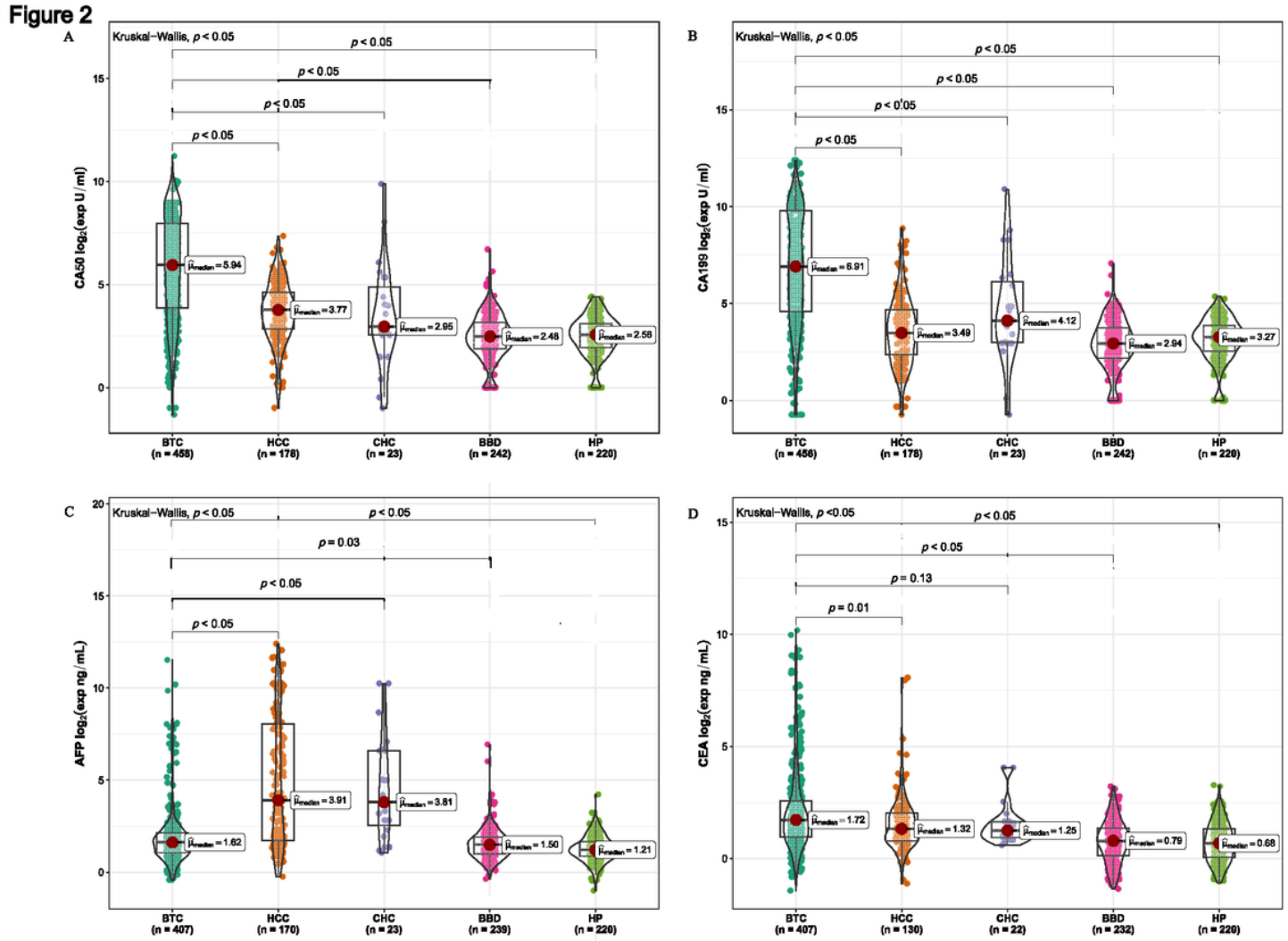

Figure 2

Differential expression levels of four tumor markers in BTC, HCC, CHC, BBD, and HP groups. (A) Levels of serum CA50 in different groups; (B) Levels of serum CA19-9 in different groups; (C) Levels of serum AFP in different groups; (D) Levels of serum CEA in different groups.

Note: biliary tract cancer (BTC); hepatocellular carcinoma (HCC); combined hepatocellular-cholangiocarcinoma (CHC); benign biliary-liver diseases (BBD); healthy people (HP); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); α-fetoprotein (AFP); carcinoembryonic antigen (CEA);

Figure 3

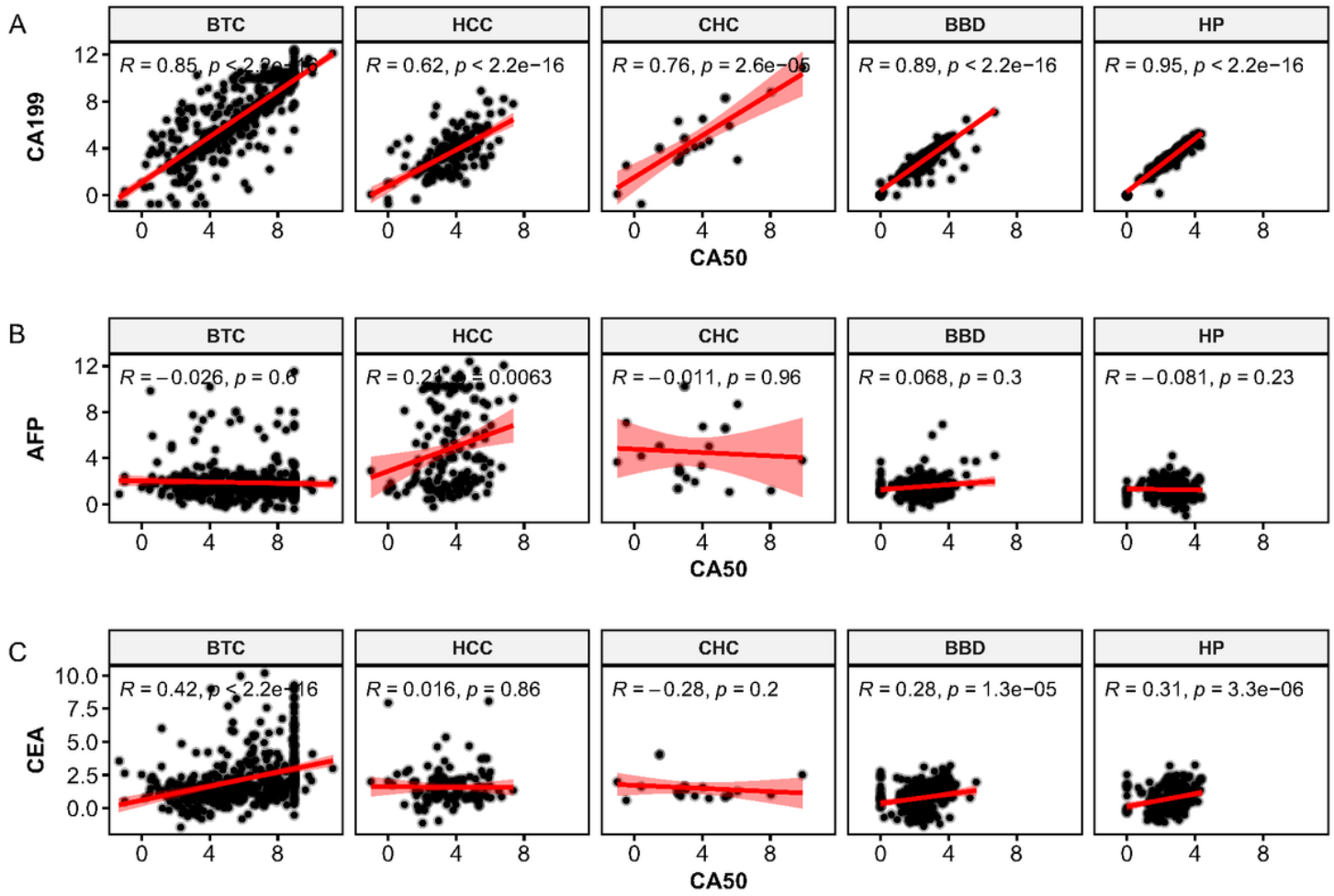

Figure 3

Correlation analysis between serum CA50 and other tumor markers. (A) Correlation of CA50 *vs.*CA19-9; (B) Correlation of CA50 *vs.* AFP; (C) Correlation of CA50 *vs.*CEA.

Note: biliary tract cancer (BTC); hepatocellular carcinoma (HCC); combined hepatocellular-cholangiocarcinoma (CHC); benign biliary-liver diseases (BBD); healthy people (HP); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); α-fetoprotein (AFP); carcinoembryonic antigen (CEA);

Figure 4

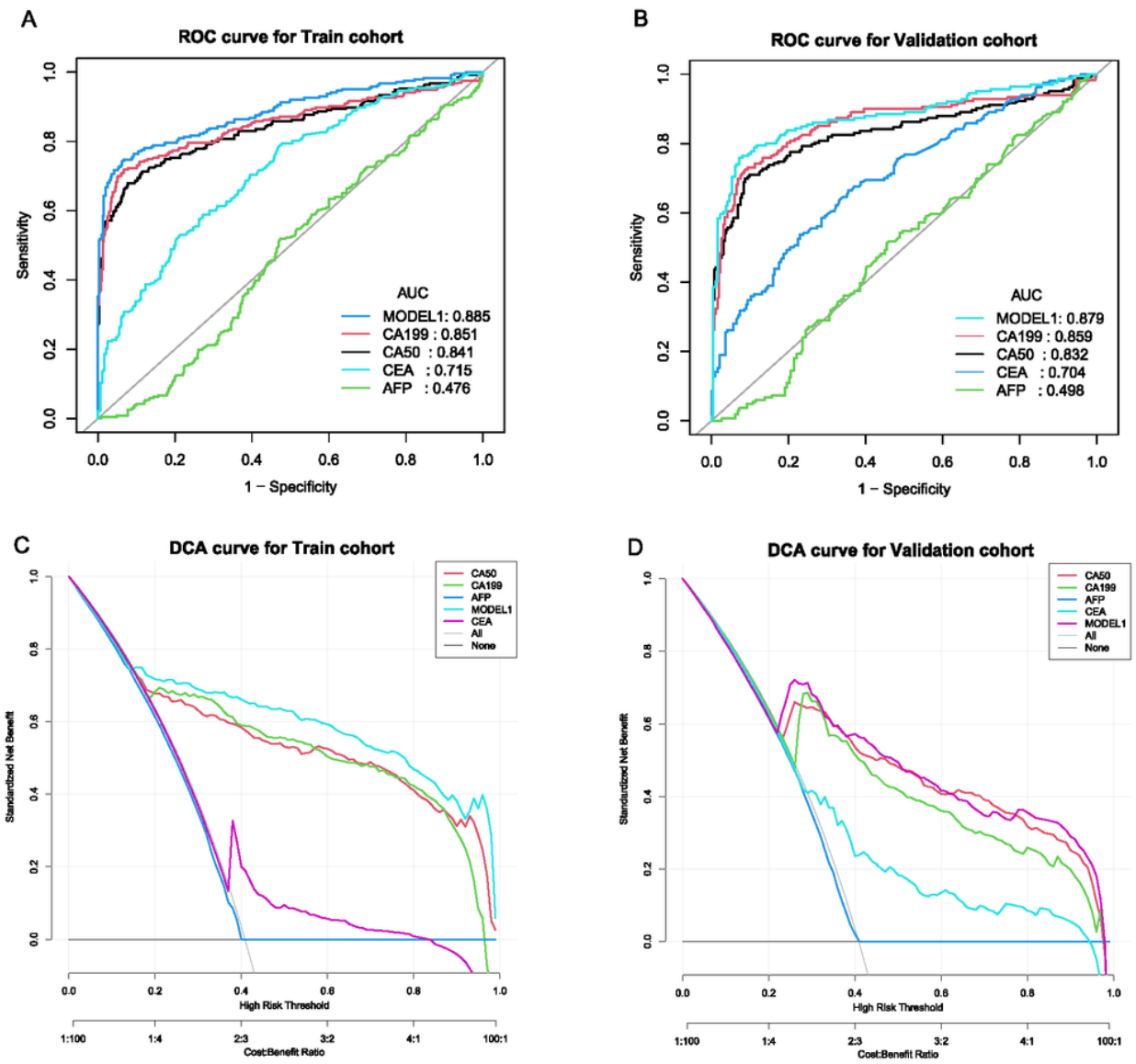

Figure 4

ROC results of different tumor markers and the model in the diagnosis of BTC. (A) ROC results of BTC *vs.* HCC+ HCC+ BBD+ HP in the train cohort; (B) ROC results of BTC *vs.*HCC+ HCC+ BBD+ HP in the validation cohort; (C) DCA results of BTC *vs.*HCC+ HCC+ BBD+ HP in the train cohort; (D) DCA results of BTC *vs.* HCC+ HCC+ BBD+ HP in the validation cohort.

Note: receiver operating characteristic curve (ROC); area under curve (AUC); decision curve analysis (DCA); biliary tract cancer (BTC); hepatocellular carcinoma (HCC); combined hepatocellular-cholangiocarcinoma (CHC); benign biliary-liver diseases (BBD); healthy people (HP); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); α-fetoprotein (AFP); carcinoembryonic antigen (CEA); MODEL 1 (CA50 + CA19-9 + AFP);

Figure 5

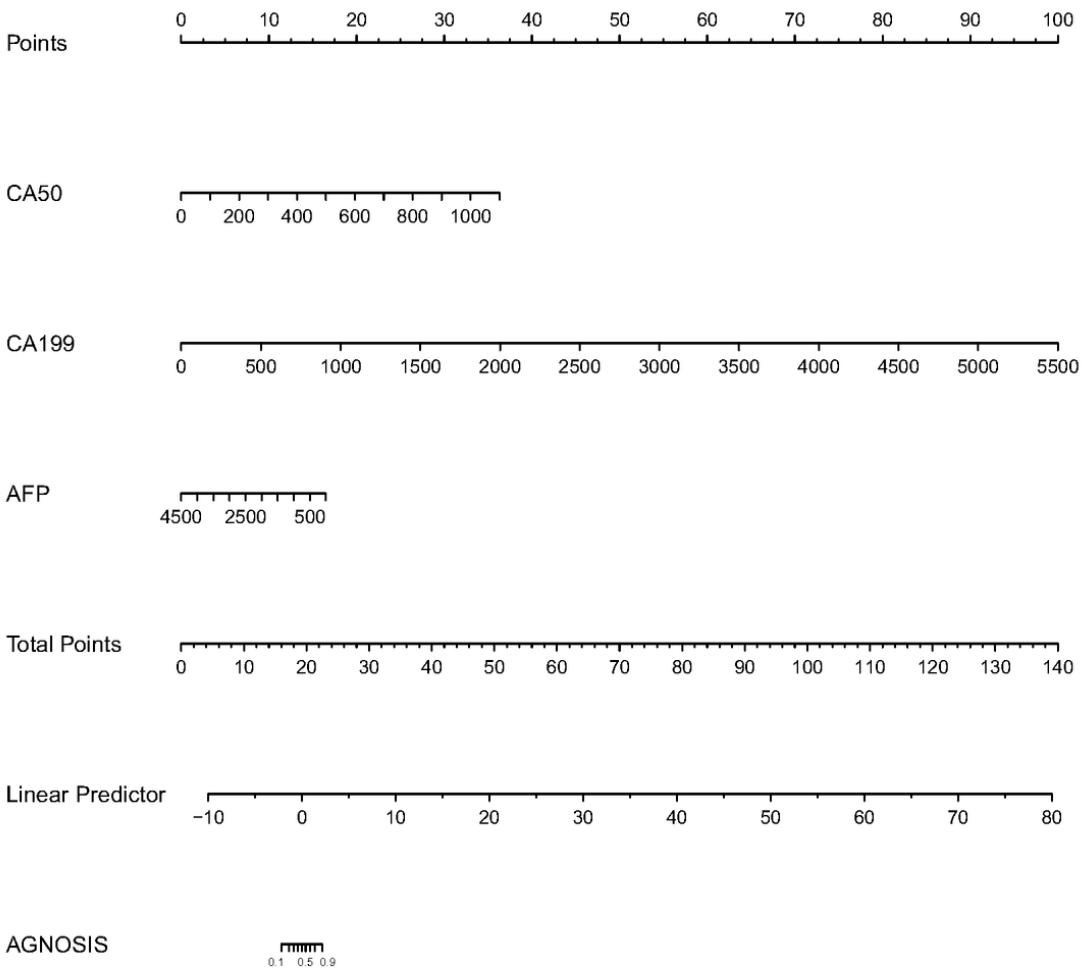

Figure 5

The nomogram of the MODEL 1 for BTC *vs.* HCC+ HCC+ BBD+ HP

Figure 6

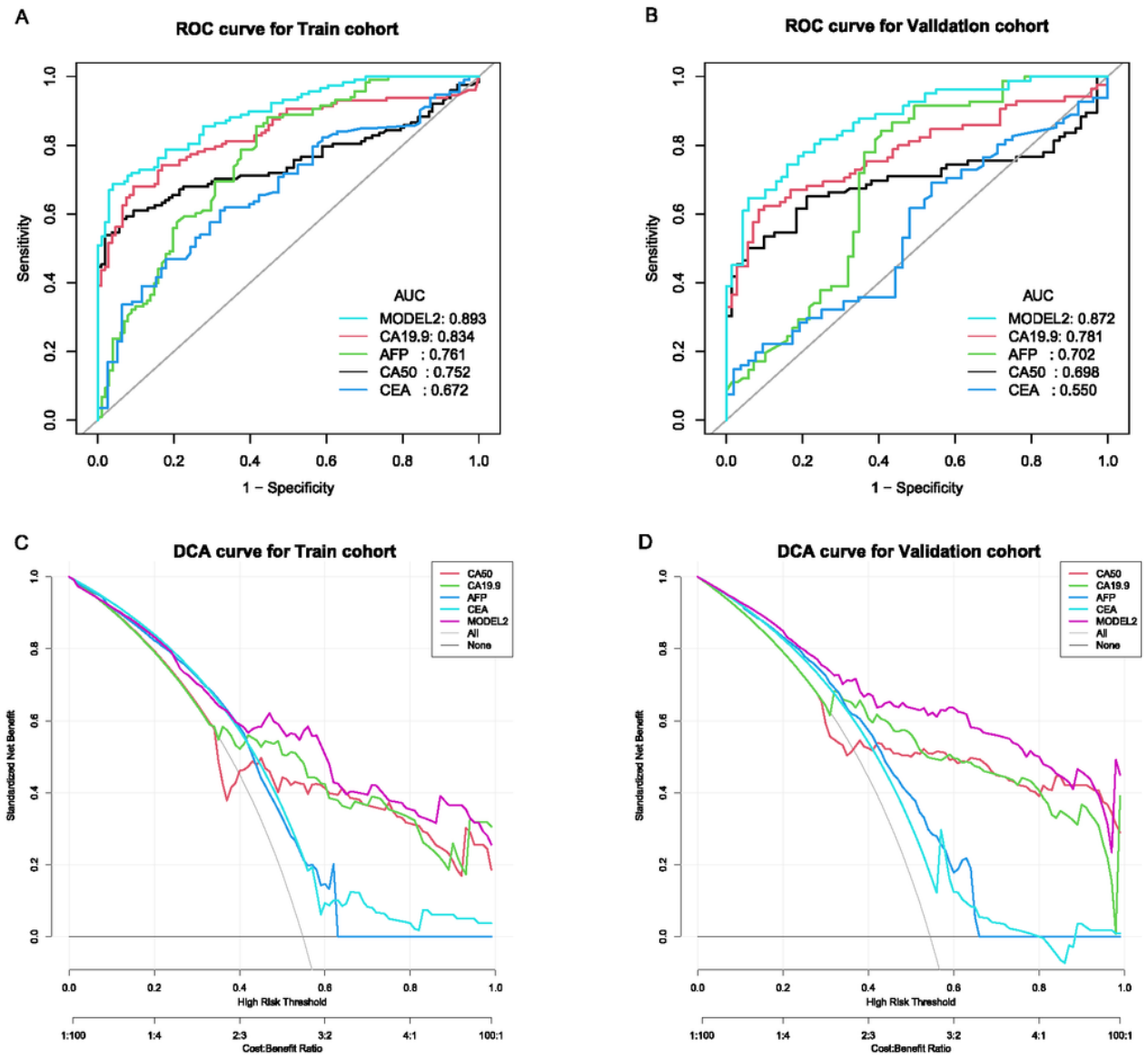

Figure 6

ROC results of different tumor markers and the model in the diagnosis of iCCA. (A) ROC results of iCCA *vs.* HCC in the train cohort; (B) ROC results of iCCA *vs.*HCC in the validation cohort; (C) DCA results of iCCA *vs.* HCC in the train cohort; (D) DCA results of iCCA *vs.* HCC in the validation cohort.

Note: receiver operating characteristic curve (ROC); area under curve (AUC); decision curve analysis (DCA) intrahepatic cholangiocarcinoma (iCCA); hepatocellular carcinoma (HCC); carbohydrate Antigen 50 (CA50); carbohydrate antigen 19-9 (CA19-9); α-fetoprotein (AFP); carcinoembryonic antigen (CEA); MODEL 2 (CA50 + CA19-9 + AFP);

Figure 7

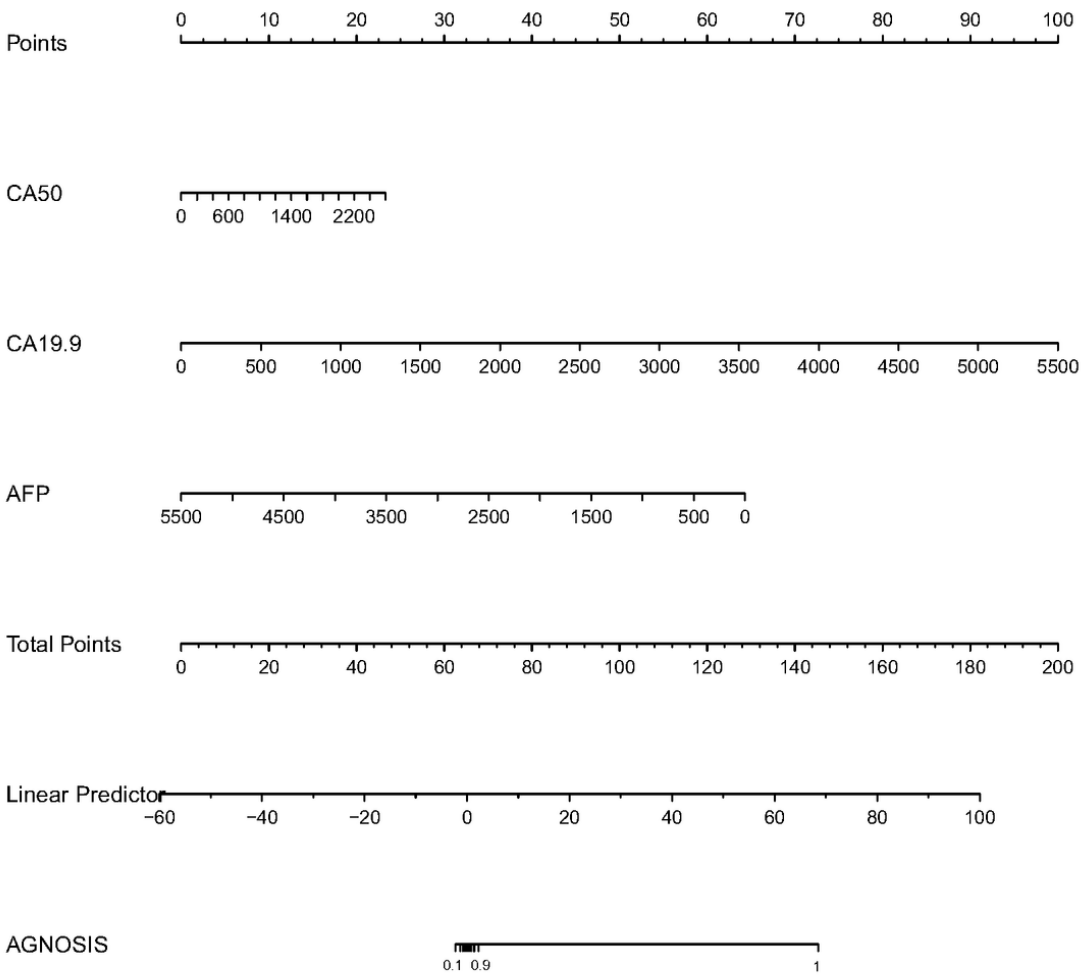

Figure 7

The nomogram of the MODEL for iCCA *vs.* HCC.

Supplementary Files

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- SupplementaryFigure3.pdf
- SupplementaryTable123.docx