A comparison of clinical pathologic characteristics between alpha-fetoprotein negative and positive liver cancer patients from Eastern and Southern China

Xiaowei Chi

The Affiliated Hospital of Shandong University of Traditional Chinese Medicine

Liejun Jiang

The People's Hospital of Guangxi Zhuang Autonomous Region

Yulin Yuan

The People's Hospital of Guangxi Zhuang Autonomous Region

Xinyan Huang

The People's Hospital of Guangxi Zhuang Autonomous Region

Xuemei Yang

The People's Hospital of Guangxi Zhuang Autonomous Region

Steven Hochwald

Roswell Park Cancer Institute

Jie Liu

Jinan Central Hospital

Huayi Huang (✉ Henry.Huang@Mindray.com)

Roswell Park Cancer Institute

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Abstract

Background

Alpha-fetoprotein (AFP) is a biomarker used in clinical management of hepatocellular carcinoma (HCC), however, approximately 40% of HCC patients do not present with elevated serum AFP levels. This study aimed to investigate the clinical and pathologic characteristics between AFP positive and negative primary liver cancer (PLC) patients to allow for improved clinical management and prognostication of the disease.

Methods

This study observed a cohort of PLC patients from Eastern and Southern China with comparisons of the clinical and pathologic features between serum AFP positive and negative patient groups; patients with decompensated hepatic cirrhosis, those with chronic hepatitis B, and hepatitis B virus (HBV) asymptomatic carrier patients were used as controls. Data included the laboratory results, pathology diagnosis, clinical staging and scores were obtained from routine clinical diagnostic methods.

Results

Patients with HCC, larger tumor sizes, liver cancer with hepatic cirrhosis, portal vein thrombosis, metastasis, high Child-Pugh score, high Barcelona-Clínic Liver Cancer (BCLC) stage, and advanced clinical stage had significantly higher serum AFP levels. Also, patients with HBsAg and HBeAg positive, high HBV DNA levels had significantly higher serum AFP levels. Patients with high serum AFP levels had higher protein induced by vitamin K absence or antagonist-II (PIVKA-II), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alpha-L-fucosidase (AFU), gamma-glutamyl transpeptidase (γ -GT), γ -GT /ALT, direct bilirubin (DBIL), indirect bilirubin (IDBIL), fibrinogen, and D-dimer levels. Serum AFP status was inversely associated with serum carcinoembryonic antigen (CEA). Patients with AFP positive had higher white blood cells (WBC), neutrophil, monocyte, and platelet count and neutrophil to lymphocyte ratio (NLR).

Conclusions

There are significant differences in clinical pathologic characteristics between AFP positive and negative PLC patients which may be helpful for the management and prognostication of the disease.

Introduction

Primary liver cancer (PLC) is a common malignancy in Asia [1]. About 364,800 new cases of liver cancer were diagnosed in China, resulting in about 318,800 deaths in 2014 [2]. The incidence of liver cancer in

China is mainly related to hepatitis B virus infection, hepatic fibrosis, and biliary cirrhosis. Treatments for liver cancer include surgical resection, interventional embolization, radiofrequency ablation, liver transplantation, and chemotherapy. However, the postoperative recurrence and metastases rates remain high and the 5 years survival rate is still very low [3, 4]. The bottle neck for long-term survival improvement is the inability for the diagnosis of early stages of the disease when the tumor size is under 3 cm. Liver cancer screening in high-risk populations relies on serum tumor markers and ultrasonography. For serum biomarkers, the alpha-fetoprotein (AFP) is commonly used as a first line screening tool recommended by guidelines worldwide and is in clinical practice because of easy accessibility, non-invasive nature, and low cost. Serum AFP concentrations >400 ng/ml is considered as reliable for supporting the diagnosis of hepatocellular carcinoma. However, about 30-40% of liver cancers are AFP-negative (< 20ng/mL) [5, 6]. The mechanism of AFP repression is unknown, it is hypothesized that silencing of *AFP* promoters because of mutation or promoter inhibition is one of the reasons [7–9]. Liver cancer patients with AFP negative or weak positive serum tests are usually consistent with the characteristics of high differentiated cancers. Although a negative serum AFP may be associated with favorable liver cancer outcomes, it results in a miss diagnosis of liver cancer in general [10, 11]. Efforts focusing on identifying new liver cancer serum biomarkers for risk evaluation, early diagnosis, recurrence, and prognosis prediction have been extensively studied, however, no new biomarkers are satisfactory thus far [12–16]. A study found that high neutrophil-lymphocyte ratio (NLR) was associated with low AFP expression in patients with hepatitis B virus (HBV) related HCC, therefore, NLR could be a potential clinical marker of the pathogenesis of AFP negative liver cancer [17]. Another study found that fibrinogen to pre-albumin ratio was gradually increased alongside with the development of AFP-negative liver cancer and positively correlated with tumor size and Barcelona Clinic Liver Cancer stages (BCLC) [18]. Thus, we hypothesize that serum biomarkers would vary between AFP-positive and negative liver cancer patients and could be indicators of liver cancer pathogenesis and possibly useful in clinical management and prognostication. In this study, we analyzed the laboratory parameters and clinical characteristics between AFP-positive and negative liver cancer patients from Eastern and Southern China to better understand the pathogenesis of the disease, improving the management, and prognostication.

Patients And Methods

Patients

This study retrospectively analyzed a total of 1047 primary liver cancer patients from both Eastern and Southern China including 992 with hepatocellular carcinoma (HCC, 861 males, 131 females, ages from 23 to 89 years and 20 to 83 years, respectively); 53 with cholangiocarcinoma (CCA, 44 males and 9 females, ages from 29 to 78 years and 41 to 72 years, respectively); 2 with hepatocellular-cholangiocarcinoma (H-ChC, 1 male and 1 female, age 42 and 63 years, respectively) from 2016 to 2020. The diagnosis of liver cancer followed the 2019 Chinese clinical guidelines for the management of hepatocellular carcinoma: updates and insights [19,20]. All liver cancer patients met pretreatment requirement, patients transferred from other hospitals were also excluded. In addition, 121 patients with decompensated hepatic cirrhosis (DHC, 85 males, 36 females, ages from 30 to 86 years and 40 to 85

years, respectively), 114 with chronic hepatitis B (CHB, 80 males, 34 females, ages from 14 to 72 years and 21 to 68 years, respectively), and 127 with hepatitis B virus asymptomatic carriers (AsC, 68 males, 59 females, ages from 5 to 67 years and 25 to 80 years, respectively) were used as control groups. Diagnosis of DHC followed the EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis [21], and diagnosis of CHB virus infection followed the EASL Clinical Practice Guidelines: Management of chronic hepatitis B virus infection and AASLD 2018 hepatitis B guidance [22,23]. This study was approved by the Institute Review Board (Ethics Committee) of The Affiliated Hospital of Shandong University of Traditional Chinese Medicine and The People's Hospital of Guangxi Zhuang Autonomous Region for the collection of patients' information. Patients' demographics are listed in Supplementary Table S1.

Chemiluminescence immunoassay of serum biomarkers

Serum AFP, carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), and cancer antigen 199 were analyzed on the Cobas e801 platform (Roche, Rotkreuz, Switzerland) and UniCel DxI 800 Access (Beckman Coulter, Brea, California, USA); Protein induced by vitamin K absence or antagonist-II (PIVKA-II) was analyzed on the ARCHITECT i2000SR platform (Abbott Park, Illinois, USA); HBsAg and HBeAg were analyzed on the EasyCuta platform (PerkinElmer, Taicang, Suzhou, China) following the manufacturers' instruction and laboratory standard operating procedures. Serum AFP level lower than 20 ng/mL was defined as AFP negative, while it was defined as AFP positive if the concentration was higher than 20 ng/mL.

Clinical chemistry analysis

Serum homocysteine (Hcy), ferritin (SF), alanine transaminase (ALT), g-glutamyl transpeptidase (g-GT), aspartate aminotransferase (AST), alpha-L-fucosidase (AFU), direct bilirubin (DBIL), indirect bilirubin (IBIL), total protein (TP), and albumin (ALB) were analyzed on the AU5800 platform (Beckman Coulter, Brea, California, USA) following the manufacturer's instruction and laboratory standard operating procedures.

Hematology analysis

Hematology analysis of blood cells (complete blood count and differentiation) were performed on the XN 9000 hematology analyzer (Sysmex, Kobe, Japan) and BC 6900 hematology analyzer (Mindray, Shenzhen, China) following the manufacturers' instruction and laboratory standard operating procedures.

Coagulation analysis

Prothrombin time (PT), fibrinogen, and D-dimer were analyzed using the STA-R Evolution (Diagnostic Stago S.A.S., Asnières sur Seine Cedex, France) following the manufacturer's instruction and laboratory standard operating procedures. The international normalized ratio (INR) was automatically calculated by the analyzer.

Hepatitis B virus DNA detection

Hepatitis B virus (HBV) DNA was detected on the C1000 Touch Thermocycler (Bio-Rad, Hercules, California, USA) by using the real time polymerase chain reaction (PCR) technique following the manufacturer's instruction and laboratory standard operating procedures. The cut-off value for DNA levels for analysis was set at 500 IU/mL.

Pathology diagnosis

The tumor histologic types and differentiation were characterized by pathology examination of routine pathologic diagnosis from surgical tissues.

Child-Pugh scoring and BCLC staging classification of hepatocellular carcinoma

The Child-Pugh Scoring was performed according to the scoring system [24]. The BCLC staging classification of hepatocellular carcinoma was used in routine clinical management [25].

Statistical analysis

All data was analyzed using the SPSS version 26 and GraphPad Prism version 9 software. Since the data sets were skewed, thus, nonparametric tests (Mann-Whitney U Test, Kruskai-Wallis H Test, Spearman Test, and Pairwise comparison) were used for the analysis. A p value <0.05 was considered significant.

Results

Normality test result of data set

The statistical analysis indicated that the data was skewed. The distribution of serum AFP in patient groups is shown in Supplementary Figure S1. Most patients' AFP levels were at the lower end (left in the histogram of Fig. S1).

Comparison of serum AFP levels among patient groups

Table 1 lists the comparison of serum AFP levels among patient groups using the nonparametric method. Statistical results of pairwise comparison are shown in Supplementary Figure S2 A. The results show that serum AFP levels in HCC and CCA patients are significantly higher than in AsC patients ($p < 0.0001$ for both), AFP levels in HCC patients are significantly higher than in CCA, CHB, and DHC patients ($p < 0.0001$ for all) (Figure S2 A).

Association between serum AFP levels and clinical pathologic features in liver cancer patients

Serum AFP levels in patients with tumor size ≥ 10 cm were significantly higher than that of < 10 cm ($p = 0.000$); patients with liver cirrhosis had higher AFP levels ($p = 0.000$); AFP levels in HCC patients were significantly higher than that of CCA patients ($p = 0.000$); Patients with portal vein thrombosis had higher

AFP levels ($p=0.000$); patients who had metastasis also had higher AFP levels ($p=0.000$); AFP levels in patients with large tumors were significantly higher than those with diffuse and single nodule tumors ($p=0.000$); high Child-Pugh score patients had higher AFP levels than those with low scores ($p=0.000$); patients with high BCLC stage had higher AFP levels than those with low stages ($p=0.000$); similarly, patients with more advanced clinical stages had higher AFP levels than that of lower stages ($p=0.000$). There was no significant correlation between tumor differentiation and serum AFP levels ($p=0.069$). Table 2. Supplementary Figure S2 B-E show the pairwise comparison (only showing the comparison with $p < 0.05$).

Association between serum AFP status and hepatitis B biomarkers in liver cancer patients

Patients with HBsAg and HBeAg positive and HBV DNA amount > 500 IU/mL had significantly higher serum AFP levels ($p=0.000$ for all). Table 3.

Association between serum AFP status and other tumor markers in liver cancer patients

Patients with high serum AFP levels had higher serum PIVKA-II ($p=0.000$). There was a negative correlation between serum AFP and CEA levels ($p=0.031$). There is no significant correlation between serum AFP and CA-125 and CA-199 levels ($p=0.474$ and 0.183 , respectively). Supplementary Table S2. The Overlay Scatter of serum AFP levels with PIVKA-II, CEA, CA-125, and CA-199 are shown in Figure 1.

Association between serum AFP status and liver function biomarkers in liver cancer patients

Patients with high serum AFP levels had higher serum alanine aminotransferase (ALT), g-glutamyl transpeptidase (g-GT), aspartate aminotransferase (AST), alpha-L-fucosidase (AFU) activities ($p=0.000$ for all), and higher g-GT/ALT ratio ($p=0.007$). Patients with high serum AFP levels also had higher serum direct bilirubin (DBIL) and indirect bilirubin (IBIL) levels ($p=0.000$ and $p=0.025$, respectively) and fibrinogen ($p=0.008$) as well as D-Dimer ($p=0.000$). There was no significant correlation between serum AFP levels and serum homocysteine (Hcy, $p=0.445$), ferritin (SF, $p=0.828$), total protein (TP, $p=0.166$), and albumin (ALB, $p=0.336$). The prothrombin time did not show significant different either ($p=0.070$) between AFP negative and positive patients. Table 4.

Association between serum AFP status and hematology parameters in liver cancer patients

Table 5 shows that serum AFP status is positively associated with white blood cell (WBC) count, neutrophils, monocytes, and platelets ($p=0.006$, 0.001 , 0.035 , and 0.012 , respectively). Serum AFP positive patients have lower lymphocytes than negative patients ($p=0.001$), thus, the neutrophils to lymphocytes ration (NLR) is higher in serum AFP positive patients ($p=0.001$).

Discussion

AFP is a major plasma protein produced by the yolk sac and the liver during fetal

life. AFP expression in adults is often associated with hepatoma or teratoma [26].

The underlying mechanism of AFP expression status in liver cancer and its role in the development and prognosis is still unclear; promoter polymorphism or silencing of *AFP* gene is believed to be the major reason [27,28]. Understanding the clinical pathologic characteristics of liver cancer patients with low and high serum AFP could be helpful for clinical management and prognostication of this deadly disease.

Serum AFP status was associated with tumor types

In this observation, we found that serum AFP levels in HCC patients were significantly higher than in cholangiocarcinoma. Serum AFP is a marker for HCC, and AFP levels tend to be lower in cholangiocarcinoma than in HCC [29]. Our observation was consistent with previous reports.

High serum AFP level was associated with unfavorable prognostic factors

We also found that patients with larger tumors, liver cancers with hepatic cirrhosis, portal vein thrombosis, metastasis, and massive hepatocellular carcinomas had higher serum AFP levels. These suggest that patients with higher serum AFP levels tend to have poor clinical outcome [30-33]. It is also possible that higher burden of disease exists in these patients accounting for the increased AFP levels.

Association of serum AFP levels between Child-Pugh score, BCLC stage, and clinical stage

Serum AFP levels were significantly higher in patients with high Child-Pugh score, high BCLC stage, and advanced clinical stage. Lim et al reported that AFP levels and Child-Pugh scores were associated with brain metastasis in HCC patients [34]. Liu et al reported that based on BCLC staging, serum AFP response was correlated with the efficacy of transarterial chemoembolization in HCC patients [35]. Incorporation of serum AFP into the BCLC staging system was helpful in predicting the outcome of HCC patients, and AFP positive was an independent poor prognostic factor [36]. Gomaa et al found that the addition of AFP and ascites to the BCLC staging system improved the prognostic prediction for early and intermediate stages of liver cancer patients [37].

Patients with HBsAg and HBeAg positive, and high HBV DNA levels had significantly higher serum AFP levels

Again, our observation further confirmed that serum AFP levels were associated with hepatitis B viral biomarkers positivity as reported previously [38]. Liver cancer patients with positive HBsAg and HBeAg, high levels of HBV DNA had higher serum AFP levels. This further indicates that active viral replication and chronic liver disorder and hepatocyte damage is occurring in liver cancer patients [39,40].

Association between serum AFP levels and other tumor markers

Patients with high serum AFP levels also had higher serum PIVKA-II. PIVKA-II is usually used in addition to AFP measurement for HCC diagnosis [41]. However, in this study, we found that there was a positive association between serum AFP and PIVKA-II levels in liver cancer patients. Si et al reported that

combined analyses of serum AFP and PIVKA-II increased the diagnostic performance for HBV-related HCC [42]. Surprisingly, we found that serum AFP levels were inversely associated with serum CEA levels ($p=0.031$) as seen in Supplementary Table S2 and Figure 1. Report mentioned that elevated serum AFP and CEA were found in advanced hepatocellular carcinoma with extrahepatic metastasis [43]. Gou et al found that both serum CEA and CA-199 increased but not AFP in primary adenosquamous carcinoma of the liver [44]. Serum CEA level may differ according to HCC subgroups which needs to be further investigated [45].

Association between serum AFP status and liver function, hemostatic status in liver cancer patients

As described above, serum AFP status was associated with Child-Pugh score. We further analyzed individual elements of the Child-Pugh scoring system, the ALT, AST, AFU, g-GT, g-GT/ALT ratio, DBIL, IBIL levels, fibrinogen, and D-dimer levels with serum AFP status. Results showed that patients with high serum AFP levels had higher serum ALT, AST, AFU, g-GT, g-GT/ALT ratio, DBIL, and IBIL levels, as well as fibrinogen and D-dimer levels. These results further confirmed the clinical usage of Child-Pugh scoring system for liver cancers. Elevated fibrinogen and D-dimer levels may suggest a potential deep venous thrombosis, or other complications in advanced tumors including liver cancer.

Association of serum AFP status with hematology parameters

Our observation found that serum AFP status was positively associated with peripheral blood WBC, neutrophils, monocytes, platelets, and the neutrophils to lymphocytes ratio (NLR). These parameters may suggest that an inflammatory or infection condition occurred in patients with high serum AFP levels, since the AFP status was associated with unfavorable clinical pathologic features as described above. Shiraki et al reported that elevated NLR is predictive of a poor survival in patients with primary HCC showing normal AFP levels [46]. Another report on a systemic review of the literature showed that NLR and albumin in HCC predicted survival better than the conventional AFP [47]. Our finding on NLR was opposite to that of Huang et al [18].

Conclusions

In summary, the results especially the laboratory parameters observed in our study in AFP negative liver cancer patients could be used for diagnosis and prognostic evaluation of these patients. HBV DNA viral load and hematologic indices are especially new parameters in associating with serum AFP status in liver cancers.

Abbreviations

HCC, hepatocellular carcinoma; PLC, primary liver cancer; CCA, cholangiocarcinoma; H-ChC, hepatocellular-cholangiocarcinoma; DHC, decompensated hepatic cirrhosis; CHB, chronic hepatitis B; AsC, hepatitis B virus asymptomatic carrier; PVT, portal vein thrombosis; MHC, massive hepatocellular carcinoma; BCLC, Barcelona clinic liver cancer staging; CEA, carcinoembryonic antigen; PIVKA-II, protein

induced by vitamin K absence or antagonist-II; CA-125, cancer antigen 125; CA-199, cancer antigen 199; Hcy, homocysteine; SF, serum ferritin; ALT, alanine aminotransferase; γ -GT, γ -glutamyl transpeptidase; AST, Aspartate aminotransferase; AFU, alpha-L-fucosidase; DBIL, direct bilirubin; IBIL, indirect bilirubin; TP, total protein; ALB, albumin; PT, prothrombin time; INR, international normalized ratio; WBC, white blood cell count; Neu, neutrophil count; Neu%, percentage of neutrophils; Lym, lymphocyte count; Lym%, percentage of lymphocytes; NLR, neutrophil to lymphocyte ratio; Mon, monocyte count; Mon%, percentage of monocytes.

Declarations

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Authors' contributions

Xiaowei Chi: collected clinical and pathologic data; **Liejun Jiang:** collected data and data analysis; **Yulin Yuan:** collected data; **Xinyan Huang:** collected data; **Xuemei Yang:** collected data; **Steven Hochwald:** discussion and clinical instruction; **Jie Liu:** project organization; **Huayi Huang:** conceived the study and writing.

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Availability of data and materials

Data is available by contacting Huayi Huang at Henry.Huang@Mindray.com.

Declarations

Ethics approval and consent to participate

This study was approved by the Institute Review Board (Ethics Committee) of The Affiliated Hospital of Shandong University of Traditional Chinese Medicine and The People's Hospital of Guangxi Zhuang Autonomous Region for the collection of patients' information. The study was waived by the ethics committee/institutional review board of The Affiliated Hospital of Shandong University of Traditional Chinese Medicine (approval No. 2021 (025)) and The People's Hospital of Guangxi Zhuang Autonomous Region (approval No. KY-LW-2021-6) for an informed consent because the data collection was conducted retrospectively.

All methods used in this study were performed in accordance with the relevant guidelines and regulations in routine clinical practice.

Consent for publication

Not applicable.

Competing interests

No competing interests.

Author details

1 Department of Laboratory Medicine, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, 42 Wenhua West Rd, Jinan, Shandong 250012, China.

2 Department of Laboratory Medicine, The People's Hospital of Guangxi Zhuang Autonomous Region, 6 Taoyuan Rd, Nanning, Guangxi 530021, China.

3 Department of Nuclear Medicine, The People's Hospital of Guangxi Zhuang Autonomous Region, 6 Taoyuan Rd, Nanning, Guangxi 530021, China.

4 Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Elm and Carlton Streets, Buffalo, New York 14263, USA.

5 Department of Laboratory Medicine, Jinan Central Hospital, 105 Jiefang Rd, Jinan, Shandong 250013, China.

6 School of Medical Laboratory, Youjiang Medical University for Nationalities, No. 98 Chengxiang Road, Baise, Guangxi 533000, China.

7 Mindray North America, 800 MacArthur Boulevard, Mahwah, NJ 07430, USA.

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Tables

Table 1 Comparison of serum AFP levels among patient groups

	n	Median	P25-P75	Mean Rank	Test Statistic	<i>P</i>
HCC	992	179.4	9.25-1210.00	840.51	405.518	0.000
H-ChC	2	225.18		596.75		
CCA	53	4.62	3.03-187.66	551.54		
CHB	114	5.72	2.80-21.99	473.05		
DHC	121	4.41	2.16-11.53	380.97		
AsC	127	2.61	1.91-3.46	229.19		

HCC: hepatocellular carcinoma; CCA: cholangiocarcinoma; H-ChC: hepatocellular-cholangiocarcinoma; DHC: decompensated hepatic cirrhosis; CHB: chronic hepatitis B; AsC: hepatitis B virus asymptomatic carrier.

Table 2 Association between serum AFP levels and clinical pathologic features

		n	Median	P25-P75	Mean Rank	Test Statistic	<i>P</i>
Tumor size ^a	<10cm	601	76.22	6.48-1000.00	403.99	-7.29	0.000
(largest in diameter)	≥10cm	294	800.00	35.47-3000.00	537.96		
Liver cirrhosis ^a	No	423	92.20	5.02-1000.00	484.06	-3.523	0.000
	Yes	624	214.51	12.77-1210.00	551.08		
Histologic types ^a	HCC	992	177.80	9.25-1210.00	534.05	-5.125	0.000
	CCA	53	4.62	3.03-187.66	316.21		
PVT ^a	No	753	85.90	6.40-1000.00	486.39	-6.448	0.000
	Yes	294	800.00	36.19-1794.64	620.34		
Metastasis ^a	No	686	103.59	6.75-1000.00	495.38	-4.226	0.000
	Yes	361	415.39	15.99-1871.47	578.38		
Types of tumor ^b	Single nodule	386	30.85	4.81-800.00	429.58	92.416	0.000
	Multiple nodules	259	154.00	8.19-1210.00	509.59		
	Diffuse	144	200.44	15.89-1288.44	559.82		
	Large tumor	258	905.02	72.28-3000.00	659.73		
TD ^b	Low	48	74.91	7.68-750.92	104.66	5.348	0.069
	Medium	120	23.06	4.33-780.26	92.46		
	High	18	6.56	3.34-64.84	70.67		
Child-Pugh ^b	A	616	69.68	6.40-1000.00	472.77	47.650	0.000
	B	311	442.89	17.14-2120.40	573.48		

	C	118	1000.00	110.66-1254.27	652.20		
BCLC ^b	0	22	12.65	4.34-70.97	261.96	50.127	0.000
	A	216	33.05	4.56-511.96	315.02		
	B	226	310.60	15.97-1866.01	417.45		
	C	283	290.89	14.37-1210.00	429.75		
	D	36	1000.00	114.29-3000.00	496.32		
Clinical staging ^b	I	63	6.99	3.39-159.21	111.44	30.016	0.000
	II	39	213.21	7.31-886.00	164.81		
	III	121	114.35	6.52-1182.25	155.85		
	IV	94	887.495	21.57-1210.00	192.52		

a: Mann-Whitney U; b: Kruskai-Wallis H; PVT: Thrombosis of portal vein; Metastasis: lymph nodes or distant organ metastasis; TD: tumor differentiation; BCLC: Barcelona clinic liver cancer staging

Table 3 Association between serum AFP levels and hepatitis B biomarkers in liver cancer patients (Mann-Whitney)

		n	Median	P25-P75	Mean Rank	Z	P
HBsAg	negative	211	8.62	3.14-800.00	372.36	-7.752	0.000
	positive	814	226.91	15.18-1210.00	549.46		
HBeAg	negative	899	141.41	6.99-1123.83	501.22	-3.915	0.000
	positive	130	401.51	34.49-3000.00	610.27		
HBV DNA	≤ 500	398	108.03	6.8-1000.00	355.24	-5.107	0.000
⊗IU/mL⊗	> 500	394	515.72	29.45-1859.49	438.18		

Table 4 Association between serum AFP levels and liver function markers in patients with AFP negative and AFP positive (Mann-Whitney)

	AFP	n	Median	P25-P75	Mean Rank	Z	P
Hcy (μg/L)	-	88	15.10	12.10-20.85	87.94	-0.764	0.445
	+	93	15.60	12.40-21.80	93.89		
SF (μg/L)	-	163	556.10	261.00-1061.71	210.37	-0.217	0.828
	+	260	570.65	270.42-1188.63	213.02		
ALT (U/L)	-	348	32.00	20.00-53.00	451.98	-5.344	0.000
	+	696	42.00	27.00-67.00	557.76		
γ-GT (U/L)	-	348	78.00	40.25-163.00	427.60	-7.191	0.000
	+	696	142.00	67.25-268.75	569.95		
γ-GT/ALT	-	348	1.32	0.54-3.11	558.34	-2.716	0.007
	+	696	0.94	0.29-3.31	504.58		
AST (U/L)	-	349	41.00	27.00-63.00	404.51	-9.014	0.000
	+	697	64.00	39.50-124.50	583.08		
AFU (U/L)	-	331	27.00	22.10-34.00	373.54	-9.883	0.000
	+	673	36.30	27.10-50.25	565.92		
DBIL (μmol/L)	-	347	4.90	3.40-9.90	470.33	-3.911	0.000
	+	696	6.45	4.00-13.98	547.76		
IBIL (μmol/L)	-	347	11.40	7.60-17.50	492.41	-2.240	0.025
	+	696	12.40	8.30-20.68	536.75		
TP (g/L)	-	348	67.05	62.30-72.20	503.26	-1.385	0.166
	+	694	67.30	62.70-72.93	530.65		
ALB (g/L)	-	348	35.90	30.93-40.60	535.19	-0.961	0.336
	+	696	35.10	30.80-39.90	516.16		
PT (sec)	-	347	14.20	13.30-15.30	496.19	-1.812	0.070
	+	692	14.40	13.60-15.40	531.94		
INR	-	247	1.13	1.05-1.23	399.52	-0.678	0.498
	+	568	1.14	1.06-1.24	411.69		
Fibrinogen		246	3.11	2.52-4.30	374.54	-2.668	0.008
		569	3.48	2.67-4.53	422.46		

D-Dimer	228	0.89	0.42-2.41	338.29	-3.716	0.000
	539	1.34	0.66-2.92	403.34		

AFP (-): <20µg/L; AFP (+): ≥20µg/L; Hcy: homocysteine; SF: serum ferritin; ALT: alanine aminotransferase; g-GT: g-glutamyl transpeptidase; AST: Aspartate aminotransferase; AFU: alpha-L-fucosidase; DBIL: direct bilirubin; IBIL: indirect bilirubin; TP: total protein; ALB: albumin; PT: prothrombin time; INR: international normalized ratio

Table 5 Association between serum AFP levels and hematology parameters (Mann-Whitney)

	AFP	n	Median	P25-P75	Mean Rank	Z	P
WBC	-	349	6.04	4.53-7.89	487.43	-2.767	0.006
	+	698	6.49	5.00-8.39	542.28		
Neu	-	349	3.62	2.55-5.52	478.07	-3.475	0.001
	+	698	4.19	3.02-6.00	546.97		
Neu%	-	349	64.00	55.85-73.30	479.40	-3.375	0.001
	+	698	67.00	59.00-74.40	546.30		
Lym	-	349	1.40	0.98-1.88	530.18	-0.468	0.640
	+	698	1.37	1.00-1.86	520.91		
Lym%	-	349	25.50	15.80-33.00	566.38	-3.207	0.001
	+	698	22.00	15.00-29.15	502.81		
NLR	-	349	2.45	1.70-4.68	480.47	-3.293	0.001
	+	698	3.05	2.04-4.93	545.76		
Mon	-	349	0.47	0.33-0.65	496.12	-2.110	0.035
	+	698	0.50	0.37-0.69	537.94		
Mon%	-	349	8.00	6.00-10.00	521.48	-0.191	0.849
	+	698	8.00	6.10-10.00	525.26		
PLT	-	249	168	126-227	379.62	-2.502	0.012
	+	572	192	127-260	424.66		

AFP (-): <20µg/L; AFP (+): ≥20µg/L; WBC: white blood cell count; Neu: neutrophil count; Neu%: percentage of neutrophils; Lym: lymphocyte count; Lym%: percentage of lymphocytes; NLR: neutrophil to lymphocyte ratio; Mon: monocyte count; Mon%: percentage of monocytes; PLT: platelet count.

Figures

Figure 1

The overlay scatter of serum AFP levels with other tumor markers (PIVKA-II, CEA, CA-125, and CA-199) Figure 1 shows the association between serum AFP levels and PIVKA-II, CEA, CA-125, and CA-199 in HCC patients using the overlay scatter plot, a p value is displayed.

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