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Original Article

Role of AFP, AFP-L3, PIVKA-II, and CA199 in diagnosis and prognosis evaluation of liver cancer

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Abstract: This study aimed to explore the role of alpha fetoprotein (AFP), AFP-L3, PIVKA-II, and carbohydrate antigen 199 (CA199) in the diagnosis and prognosis of liver cancer (LC). A total of 86 patients with primary LC were categorized as the liver cancer group (LCG), 85 non-LC patients with chronic hepatitis B as the non-LC group (NLCG), and 80 healthy subjects as the control group (CG). The expression levels of AFP, PIVKA-II, and CA199 among the three groups were compared. Kaplan-Meier curve was adopted to analyze the relationship between AFP, PIVKA-II, CA199, and the prognosis of patients with LC. The serum expression levels of AFP, PIVKA-II, and CA199 were different among the three groups ($P < 0.001$). The test with all four factors in combination had the best diagnostic efficacy. The diagnosis with PIVKA-II had the highest specificity. The patients were divided into the high expression group (HEG) and low expression group (LEG) by the expression levels of AFP, AFP-L3, PIVKA-II, and CA199. It showed that the 5-year survival rate in HEG was lower than that in LEG ($P < 0.05$). Cox multiple-factor analysis showed that tumor size, differentiation, TNM stage, AFP-L3, and PIVKA-II were the independent risk factors of prognosis. The test with AFP, AFP-L3, PIVKA-II, and CA199 in combination had a better diagnostic value. High expression of AFP, AFP-L3, PIVKA-II, and CA199 is related to poor prognosis of patients with LC.

Keywords: AFP, AFP-L3, PIVKA-II, CA199, liver cancer, diagnosis

Introduction

Liver cancer (LC) is the second most common cause of cancer-related death in men. The disease can occur at all ages. The age of onset is approximately between 40 and 49 years old [1, 2]. LC is most prevalent in Asia and Africa. However, it is also rapidly becoming common in western countries due to the spread of hepatitis and the increased incidence of alcohol-related and non-alcoholic steatohepatitis-related LC [3, 4]. The pathogenesis of LC has not been clearly studied. LC is characterized by concealed pathogenesis, high degree of malignancy, high mortality, and poor prognosis. The mortality is only second to that of gastric cancer and esophageal cancer, and the recent survival rate of LC within 5 years is about 8% [5].

At present, LC diagnosis mainly depends on the clinical characteristics, imaging, and tumor markers [6]. Alpha fetoprotein (AFP) is one of the most widely used tumor markers in the clinical diagnosis of primary LC. However, the sensitivity is less than 60% [7]. AFP has three subtypes. Studies have found that AFP-L3 is mainly present in patients with primary LC. Detection of AFP subtypes improves the quasi-specificity of AFP for the diagnosis of primary LC to some extent. However, the result is still not very satisfactory. Negative AFP exists in about 30% of patients with primary LC [8]. CA199 is often used for the diagnosis of progressive and metastatic digestive tract tumors, as well as for the diagnosis of cholangiocellular carcinoma [9]. Further study indicated that abnormal prothrombin (PIVKA-II) and other factors are also expressed abnormally in LC. PIVKA-II has a good specificity in the diagnosis of primary LC [10]. However, the diagnosis of LC with the three factors in combination has not been reported.

In this study, the diagnostic value of AFP, PIVKA-II, and CA199 in combination was analyzed. Thus, a reference for the clinical diagnosis of LC is provided.

Materials and methods

Subjects of study

In total, 86 patients with primary LC in our hospital were enrolled in the LC group (LCG), 85 non-LC patients with chronic hepatitis B were classified into the non-LC group (NLCG), and 80 healthy patients were classified as the control group (CG). The subject ages ranged from 30 to 50 years. The inclusion criteria were as follows: all patients with primary LC were diagnosed with LC by pathological examination at our hospital. The patients with chronic hepatitis B met the Guidelines for Prevention and Treatment of Chronic Hepatitis B 2010. All the liver function measures were normal in the healthy subjects and no obvious disease attributes were observed. The exclusion criteria were as follows: abnormal bleeding, previous history of tumors, a medical history of hepatitis, mental or learning dysfunction, presence of a large mass combined with other cardiovascular and cerebrovascular diseases, presence of other respiratory diseases, presence of digestive tract diseases, gastrointestinal dysfunction before operation, and midway transfer to another hospital. The study was approved by the Ethics Committee of our hospital. The patients or their family members signed the informed consent form.

Test measures

The preoperative peripheral blood of patients with primary LC was collected. All blood samples were collected under fasting conditions in the morning and submitted for testing within 2 h. Serum was separated from the blood samples after centrifugation at 4000 rpm for 30 min. The level of serum PIVKA-II was determined using a chemiluminescence method. The levels of serum AFP and CA199 were quantitatively determined by an electrochemiluminescence method. The chemiluminescent analyzer was purchased from Cloud-Clone Corp, Wuhan (Art. No. SCA17.1). The electrochemiluminescence instrument was purchased from Runwell Industry Limited Company (Art. No. 725501KT).

Outcome measures

The expression levels of AFP, PIVKA-II, and CA199 were compared among the three groups. ROC was introduced to analyze the diagnostic value of AFP, PIVKA-II, and CA199 in LC. The Kaplan-Meier curve was used to analyze the relationship between AFP, PIVKA-II, and CA199, and the prognosis.

Statistical analysis

Data were analyzed using SPSS 19.0 was used. The enumeration data and the measurement data were expressed as ratios and mean ± SD, separately. The χ^2 test was performed using the comparison of ratios. LSD test was introduced for back testing. The survival curve was plotted using the Kaplan-Meier method. The diagnostic value was analyzed using ROC. Cox regression analysis was used for the risk factors of prognosis. $P < 0.05$ implied a significant difference.

Results

General information

There was a difference in the expression levels of Tbil, Dbil, ALT, and AST ($P < 0.05$). The expression levels in the CG were lower than those in the LCG and NLCG ($P < 0.001$). The expression levels in the NLCG were higher than those in the LCG ($P < 0.001$). The details of other clinicopathologic features are shown in **Table 1**.

Expression of AFP, AFP-L3, PIVKA-II, and CA199

The expression of AFP, PIVKA-II, and CA199 in the CG was lower than that in the LCG and NLCG ($P < 0.001$). The expression in the LCG was higher than that in the NLCG ($P < 0.001$) (**Table 2**).

Diagnostic value of AFP, AFP-L3, PIVKA-II, and CA199 in patients with LC and non-LC

The AUC of AFP for the differential diagnosis of LC and non-LC was 0.659, the critical value was 165.20 μg/L, the specificity was 66.28%, and the sensitivity was 58.82%. For AFP-L3, the AUC was 0.823, 6.48%, 72.09%, and 85.29%, respectively. For PIVKA-II, the AUC, critical value, specificity, and sensitivity was 0.887, 7640.00 μg/L, 90.07%, and 72.94%, respectively. For CA199, the AUC, critical value, specificity, and sensitivity was 0.813, 47.72 kU/L, 63.95%, and 82.94%, respectively. For the four factors in combination, the AUC, specificity, and sensitivity were respectively 0.978,

Table 1. General information

	Liver cancer Group (n = 86)	Non liver Cancer group (n = 85)	Control Group (n = 80)	χ^2/F	P
Sex				2.209	0.331
Man	58 (67.44)	53 (62.35)	45 (56.25)		
Woman	28 (32.56)	32 (37.65)	35 (43.75)		
Age (years)	52.15±12.85	48.68±14.27	47.83±16.84	2.042	0.132
BMI (kg/m^2)	18.07±2.59	18.42±3.03	18.97±3.25	1.936	0.147
Tumor size					
≤ 5 cm	21 (24.42)				
> 5 cm, < 10 cm	43 (50.00)				
≥ 10 cm	22 (25.58)				
Tbil (μmol/L)	28.43±3.37	106.01±44.33*	11.95±1.51*,#	315.126	< 0.001
Dbil (μmol/L)	22.09±3.46	45.86±12.64*	3.25±0.38*,#	646.809	< 0.001
ALT (U/L)	122.14±12.63	186.42±21.23*	18.64±2.45*,#	2811.512	< 0.001
AST (U/L)	128.05±13.54	369.71±25.84*	21.23±3.47*,#	9055.663	< 0.001
Differentiation [(n%)]					
I, II	54 (62.79)				
III, IV	32 (37.21)				
Tumorous Numbers [(n%)]					
Single	52 (60.47)				
Multiple	34 (39.53)				
TMN stage					
I, II	40 (46.51)				
III, IV	46 (53.49)				

Note: Tbil, total bilirubin; Dbil, direct bilirubin; ALT, glutamic-pyruvic transaminase; AST, glutamic oxalacetic transaminase; *implies P < 0.05 in liver cancer group. #implies P < 0.05 in non-liver cancer group.

Table 2. Expression of AFP, PIVKA-II, and CA199

	Liver cancer Group (n = 86)	Non liver Cancer group (n = 85)	Control Group (n = 80)	F	P
AFP (μg/L)	196.14±90.28	146.41±70.27*	14.59±4.27*,#	161.756	< 0.001
AFP-L3 (%)	8.86±3.49	5.00±1.01*	2.50±0.55*,#	185.420	< 0.001
PIVKA-II (μg/L)	11777.89±5388.91	4713.79±2212.64*	20.23±10.69*,#	251.446	< 0.001
CA199 (kU/L)	54.99±20.59	33.37±10.24*	24.02±5.74*,#	110.476	< 0.001

Note: *represents P < 0.05 in the liver cancer group. #represents P < 0.05 in the non-liver cancer group.

Table 3. Diagnostic value of AFP, PIVKA-II, and CA199 in patients with liver cancer and non-liver cancer

	AFP	AFP-L3	PIVKA-II	CA199	Combination
AUC	0.659	0.823	0.887	0.813	0.918
95% CI	0.578-0.740	0.752-0.894	0.833-0.940	0.747-0.880	0.958-0.998
cut off	165.20 μg/L	6.48%	7640.00 μg/L	47.72 kU/L	
Specificity%	66.28	65.09	90.07	63.95	89.53
Sensitivity%	58.82	85.29	72.94	82.94	92.82

89.53%, and 92.82%. Detection with the four factors in combination had the best diagnostic efficacy. The diagnosis with PIVKA-II had the highest specificity (**Table 3**; **Figure 1**).

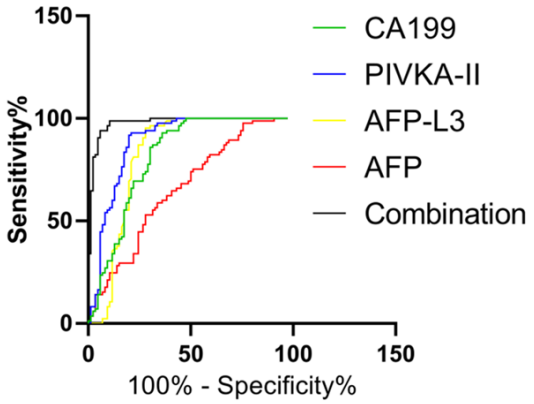

Figure 1. Diagnostic value of AFP, AFP-L3, PIVKA-I, and CA199 in patients with liver cancer and non-liver cancer. AUC (AFP) = 0.659, AUC (AFP-L3) = 0.823, AUC (PIVKA-II) = 0.887, AUC (CA199) = 0.813, AUC (combination) = 0.918.

Relationship between AFP, AFP-L3, PIVKA-II, CA199 and the prognosis of patients with LC

The patients with LC were classified into the high expression group (HEG) and low expression group (LEG) based on the expression of AFP, AFP-L3, PIVKA-II, and CA199 in peripheral blood. The median was used as the critical value. The Kaplan-Meier survival analysis results showed that the 5-year survival rate in the LEG was higher than that in the HEG ($P < 0.05$) (**Figure 2**).

Prognostic risk factor analysis

Cox multiple-factor analysis results showed that the tumor size, differentiation, TNM stage, AFP-L3 and PIVKA-II were independent prognostic risk factors.

Discussion

Early discovery and early treatment are the most important factors for the treatment of tumors clinically. CT and other imaging methods for the diagnosis of early LC have low specificity and missed diagnosis may occur. Therefore, serological tests are still an essential means of diagnosis [11, 12]. In this study, the diagnostic value of AFP, AFP-L3, PIVKA-II, and CA199 in LC was analyzed. Thus, a basis for clinical diagnosis was provided (**Table 4**).

Under normal physiological conditions, AFP is not expressed and synthesized in hepatocytes. There are low levels of AFP in the peripheral blood. However, AFP can be expressed in hepatoma cells, resulting in high levels of AFP in the peripheral blood. However, the sensitivity of AFP for early LC detection is low. High expression of AFP may also exist in hepatitis, liver cirrhosis, and other tumors. Diagnosis of LC with AFP alone has great limitations [13, 14]. Some researchers have isolated AFP-L3, which is a variant of AFP, from peripheral blood. Studies have shown that the specificity of AFP-L3 for the diagnosis of LC is much higher than that of AFP. However, the sensitivity is still not high and is only about 60% [15, 16]. The liver is an important organ for the synthesis of prothrombin. The conversion of Gla to γ-carboxyglutamic acid catalyzed by γ-glutamate carboxylase is an important process in the formation of normal prothrombin. However, a study has shown that γ-glutamate carboxylase activity in liver cells of patients with LC was decreased. As a result, the prothrombin synthesized by liver cells was unable to bind calcium ions and phospholipids. PIVKA-II is formed and has no coagulation activity [17, 18]. CA199 is a glycoprotein and s synthesized by tumor cells. It is often used in the diagnosis of pancreatic cancer. The level of CA199 in hepatobiliary carcinoma is up to 535 times the normal value [19, 20]. However, there are few reports on the diagnostic efficacy of CA199 in LC (**Table 5**).

The results of this study showed that the expression of AFP, AFP-L3, PIVKA-II, and CA199 in patients with LC was significantly higher than that in patients with non-LC. ROC analysis results showed that combined testing of AFP, AFP-L3, PIVKA-II, and CA199 had the highest diagnostic efficacy. The AUC was 0.918. The sensitivity was up to 92.82%. The specificity of PIVKA-II was lower than 90.07%. In the report by Park, et al. [21], the diagnostic value of AFP, AFP-L3, and PIVKA-II in hepatocellular carcinoma was analyzed. AFP had the highest diagnostic efficacy. The AUC was 0.751. The AUC of diagnosis with the three indexes in combination was 0.765. However, Lim, et al. [8] showed that the AUC was up to 0.877. The AUC for the diagnosis of early LC was 0.773. AFP > 400 ng/mL is often used as the critical value for LC diagnosis. Although sensitivity is improved, the diagnosis of about 30% of the patients is missed [22]. Use of CA199 improves the diagnostic efficacy of LC. However, more experimental data are needed to validate this conclusion.

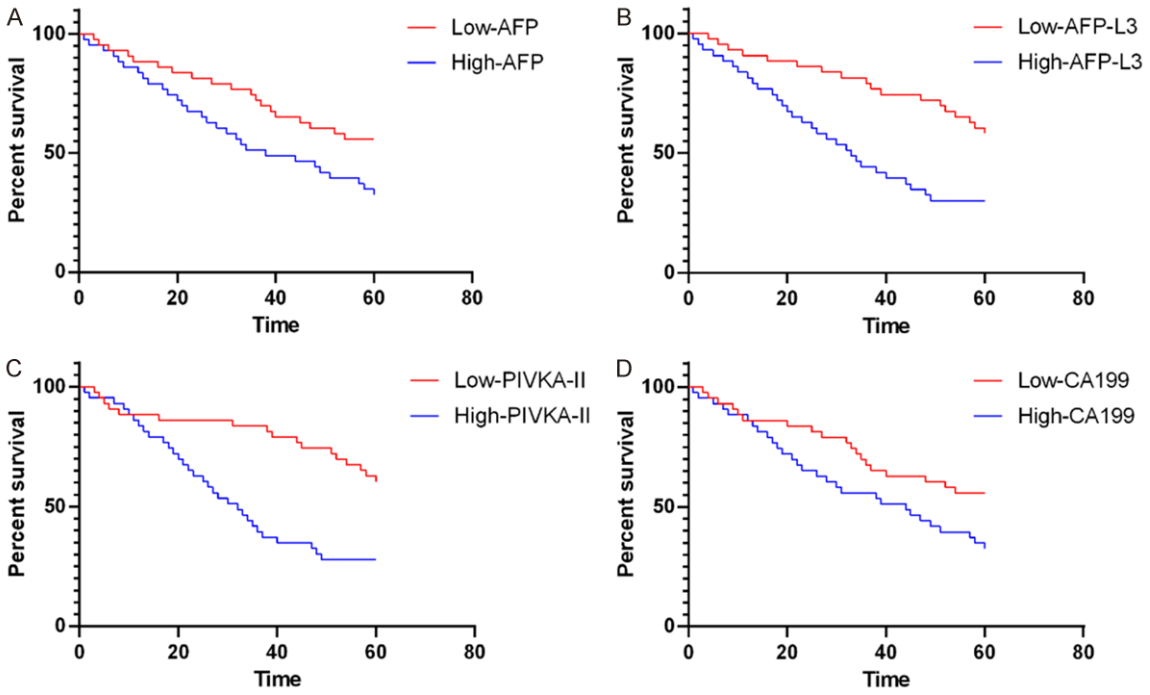

Figure 2. Relationship between AFP, AFP-L3, PIVKA-II, CA199, and prognosis. The 5-year survival rate of patients with high expression of AFP, AFP-L3, PIVKA-II, and CA199 was lower than that of patients with low expression of AFP, AFP-L3, PIVKA-II, and CA199 ($P < 0.05$).

Table 4. Assignment table

	Assignment
Sex	Man = 1, Woman = 0
Age	Continuous variable
BMI	Continuous variable
Tumor size	< 10 cm = 1; ≥ 10 cm = 0
Tbil	Continuous variable
Dbil	Continuous variable
ALT	Continuous variable
AST	Continuous variable
Differentiation	I, II = 1; III, IV = 0
Tumorous numbers	Single = 1; Multiple = 0
TMN stage	I, II = 1; III, IV = 0
AFP	Continuous variable
AFP-L3	Continuous variable
PIVKA-II	Continuous variable
CA199	Continuous variable
Prognosis	Death = 1, Survival = 0

In this study, the relationship between AFP, AFP-L3, PIVKA-II, CA199 and the 5-year survival rate of patients with LC was analyzed. The analysis results showed that the 5-year survival rate in the HEG was much lower than that in the LEG. Cox risk factor analysis results showed that high expression of AFP, AFP-L3, PIVKA-II, and CA199 was associated with poor prognosis and was dependent on risk factors of prognosis (**Table 6**). Ma et al. [23] reported that the pre-operative serum AFP level has a significant predictive value for the malignant degree and prognosis of hepatocellular carcinoma, indicating that the efficacy is better in patients with LC when there are no surgical contraindications and the serum AFP is 20 ng/ml. Wang et al. [24] reported that AFP-L3 is a risk factor for postoperative recurrence of LC. The lower the severity of clinical features, the lower is the expression of AFP-L3. PIVKA-II is also reported to be related to the prognosis of patients with hepatocellular carcinoma. AFP and PIVKA-II in combination are independent risk factors of prognosis [25]. The relationship between multiple index combination and prognosis was not analyzed in this study. A new direction for the future analysis of prognostic risk factors is thus provided. Other study research has indicated that "Alpha-fetoprotein showes the best diagnostic performance as a single biomarker for HCC. The diagnostic value of AFP was improved by combining it with PIVKA-II, but adding AFP-L3 did not contribute

Table 5. Single-factor analysis

	B	SE	Wald	df	Sig.	Exp (B)	95.0% CI	
							Lower Limit	Upper Limit
Sex	-0.111	0.302	0.135	1	0.713	0.895	0.495	1.618
Age	0.013	0.011	1.434	1	0.231	1.013	0.992	1.036
BMI	-0.107	0.053	4.150	1	0.042	0.899	0.811	0.996
Tumor size	-0.884	0.303	8.498	1	0.004	0.413	0.228	0.749
Tbil	0.106	0.040	6.991	1	0.008	1.112	1.028	1.203
Dbil	0.112	0.042	7.188	1	0.007	1.118	1.031	1.214
ALT	0.025	0.011	5.218	1	0.022	1.025	1.004	1.047
AST	0.021	0.009	5.270	1	0.022	1.021	1.003	1.039
Differentiation	-0.989	0.291	11.523	1	0.001	0.372	0.210	0.658
Tumorous Numbers	-0.615	0.290	4.490	1	0.034	0.541	0.306	0.955
TMN stage	-1.806	0.362	24.912	1	0.000	0.164	0.081	0.334
AFP	0.006	0.002	14.754	1	0.000	1.006	1.003	1.009
AFP-L3	0.189	0.040	22.879	1	0.000	1.208	1.118	1.306
PIVKA-II	0.000	0.000	7.049	1	0.008	1.000	1.000	1.000
CA199	0.031	0.008	16.918	1	0.000	1.032	1.017	1.047

Table 6. Multiple-factor analysis

	B	SE	Wald	df	Sig.	Exp (B)	95.0% CI	
							Lower Limit	Upper Limit
BMI	-0.082	0.072	1.324	1	0.250	0.921	0.800	1.060
Tumor size	-0.850	0.392	4.714	1	0.030	0.427	0.198	0.921
Tbil	0.078	0.059	1.748	1	0.186	1.081	0.963	1.212
Dbil	0.083	0.065	1.636	1	0.201	1.086	0.957	1.233
ALT	0.019	0.014	1.890	1	0.169	1.019	0.992	1.047
AST	0.049	0.054	0.847	1	0.357	1.051	0.946	1.167
Differentiation	0.000	0.000	11.439	1	0.001	1.000	1.000	1.000
Tumorous Numbers	-0.364	0.357	1.038	1	0.308	0.695	0.345	1.399
TMN stage	-1.187	0.413	8.266	1	0.004	0.305	0.136	0.685
AFP	0.002	0.002	1.553	1	0.213	1.002	0.999	1.006
AFP-L3	0.041	0.017	6.006	1	0.014	1.042	1.008	1.077
PIVKA-II	0.021	0.011	3.873	1	0.049	1.021	1.000	1.043
CA199	-0.001	0.373	0.000	1	0.198	0.999	0.481	2.076

to the ability to distinguish between HCC and non-HCC liver cirrhosis. These findings were not altered when the cut-off value of AFP and AFP-L3 was changed". In the above study, "AFP, AFP-L3, and PIVKA-II were measured in the same serum samples using microchip capillary electrophoresis and a liquid-phase binding assay on an automatic analyzer". It method is different from our study, and maybe the different methods and test results need to be further compared.

The empirical results reported herein should be considered in the light of some limitations. The study has a small sample size, and there may be errors in the experimental results. In addition, the experimental design is from a single-center, and there may be regional differences, resulting in inaccuracy of results.

In summary, combined detection of AFP, AFP-L3, PIVKA-II, and CA199 is valuable in diagnosing LC. High expression of AFP, AFP-L3, PIVKA-II, and CA199 is related to poor prognosis in LC patients.

Disclosure of conflict of interest

None.

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