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BRIEF ARTICLE

Value of α-fetoprotein in association with clinicopathological features of hepatocellular carcinoma

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Abstract

AIM: To explore the relationship between α-fetoprotein (AFP) and various clinicopathological variables and different staging system of hepatocellular carcinoma (HCC) thoroughly.

METHODS: A retrospective cohort study of consecutive patients diagnosed with HCC between January 2008 and December 2009 in West China Hospital was enrolled in our study. The association of serum AFP values with the HCC clinicopathological features was analysed by univariate and multivariate analysis, such as status of hepatitis B virus (HBV) infection, tumor size, tumor number, vascular invasion and degree of tumor differentiation. Also, patients were divided into four groups at the time of enrollment according to different cutoff values for serum value of AFP ($\leqslant$ 20 μg/L, 21-400 μg/L, 401-800 μg/L, and $\geqslant$ 801 μg/L), to compare the positive rate of patient among four groups stratified by various clinicopathological variables. And the correlation of different kinds of tumor staging systems, such as TNM, Barcelona Clinic Liver Cancer (BCLC) staging classification and China staging, were compared with the serum concentration of AFP.

RESULTS: A total of 2304 HCC patients were enrolled in this study totally; the mean serum level of AFP was 555.3 ± 546.6 μg/L. AFP levels were within the normal range (< 20 μg/L) in 27.4% (n = 631) of all the cases. 81.4% (n = 1875) patients were infected with HBV, and those patients had much higher serum AFP level compared with non-HBV infection ones (573.9 ± 547.7 μg/L *vs* 398.4 ± 522.3 μg/L, P < 0.001). The AFP level in tumors $\geqslant$ 10 cm (808.4 ± 529.2 μg/L) was significantly higher (P < 0.001) than those with tumor size 5-10 cm (499.5 ± 536.4 μg/L) and with tumor size $\leqslant$ 5 cm (444.9 ± 514.2 μg/L). AFP levels increased significantly in patients with vascular invasion (694.1 ± 546.9 μg/L *vs* 502.1 ± 543.1 μg/L, P < 0.001). Patients with low tumor cell differentiation (559.2 ± 545.7 μg/L) had the significantly (P = 0.007) highest AFP level compared with high differentiation (207.3 ± 420.8 μg/L) and intermediate differentiation (527.9 ± 538.4 μg/L). In the multiple variables analysis, low tumor cell differentiation [OR 6.362, 95%CI: 2.891-15.382, P = 0.006] and tumor size ($\geqslant$ 10 cm) (OR 5.215, 95%CI: 1.426-13.151, P = 0.012) were independent predictors of elevated AFP concentrations (AFP > 400 μg/L). Serum AFP levels differed significantly (P < 0.001) in the D stage of BCLC (625.7 ± 529.8 μg/L) compared with stage A (506.2 ± 537.4 μg/L) and B (590.1 ± 551.1 μg/L).

CONCLUSION: HCC differentiation, size and vascular invasion have strong relationships with AFP, poor differentiation and HCC size $\geqslant$ 10 cm are independent predictors of elevated AFP. BCLC shows better relationship with AFP

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and third most significant cause of cancer mortality in the world, with 5-year survival rates at a mere 7% in patients[1-3]. When patients with obvious clinical symptoms come to the hospital for treatment, the HCC has already reached the mild to advanced stages and is usually large in size. Given the subsequent rapid growth and vascular invasion, patients have to face serious progress and poor prognosis. There is no doubt that a thorough comprehension of the pathobiological features of HCC will definitely help clinicians diagnose HCC at earlier stages, thus improving patient outcomes.

Currently, the most commonly used methods for screening and diagnosing HCC are ultrasound imaging and serum α-fetoprotein (AFP) concentration measurements. AFP has been used worldwide as the golden standard compared to other serum markers, especially in poor, remote areas. However, the diagnostic value of AFP is still controversial given that its sensitivity and specificity are unstable[4,5]. Moreover, researchers have studied AFP-related parameters, such as AFP mRNA[6-8] and AFP glycoforms[9-11]. Recently, both of these AFP-related parameters have been proven to have diagnostic potential in a way, and even they are recommended as complementary tests. Nevertheless, their usage is limited due to financial and technological reasons; it is unlikely that they can replace serum AFP as the golden standard of diagnostic serum markers for hepatocellular carcinoma.

The serum AFP level also plays an important role in representing the pathobiological features of HCC identified as prognostic factors[12-14]. Some clinicians have reported that the elevation of serum AFP levels is consistent with increased tumor sizes[15-17] and that small tumors seem to excrete less AFP into the blood. They also report that there is no clear increasing tendency between tumor size and AFP levels[18]. Others have reported that highly differentiated small tumors express undetectable levels of serum AFP[14], whereas lowly differentiated tumor cells, in most cases, had AFP levels of > 400 μg/L[19]. Similarly, other factors such as tumor number[14,16] and vascular invasion[14,20-22] have been found to be correlated with serum AFP levels to a certain extent. Nonetheless, these studies failed to research the relationship between various variables thoroughly, and the sample size of some cohort studies was not large enough.

The objects of this study were to comprehensively evaluate the relationship between AFP and clinicopathological features of HCC, to compare the clinical practicality of different tumor staging systems, and to state the clinical importance of measuring AFP concentrations in cases of HCC in China.

MATERIALS AND METHODS

Ethics
Informed consent was obtained from all subjects for participation in the study and the usage of sera. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the ethical committee of the West China Hospital, Sichuan University. All participants provided their written informed consent to participate in this study.

Patient tissue
The base population consisted of 2381 consecutive patients who received a final diagnosis of HCC at the department of liver and vascular surgery, West China Hospital, Sichuan University, between January 2008 and December 2009. Of these 2381 patients, 31 were excluded from the study because of the absence of tumor size data, and 46 were excluded due to missing serum AFP level data. Therefore, a total of 2304 HCC patients were included in this study. The final diagnosis of HCC was made in two main ways according to whether the patients had a hepatectomy or not. HCC diagnosis was made by the pathological examination of the resected specimens for the 1825 patients (79.2%) who received hepatic resection or fine needle aspiration. The remaining 479 patients (20.8%) had advanced HCC with impaired liver function, with portal vein invasion or metastasis, and thus did not undergo an invasive operation. Instead, this group of patients had their HCC confirmed by a variety of combined imaging techniques, identification of a focal lesion > 2 cm in diameter in 2 imaging modalities, such as ultrasonography (US), enhanced computed tomography (CT) image, magnetic resonance imaging (MRI), and/or angiography, and showing arterial hypervascularization in at least 1 of imaging modalities. A mass lesion within a cirrhotic liver in the presence of a serum AFP level > 400 μg/L was also diagnostic[23]. For lesions < 2 cm in diameter, diagnosis of HCC required hepatitis B surface antigen (HBsAg) positive and AFP > 400 μg/L. The severity of liver dysfunction was assessed *via* the Child-Pugh classification. Serum samples were collected and stored at -70 ℃ until examined. Hepatitis B virus (HBV) infection status was determined on the basis of HBsAg, hepatitis B surface antibody, hepatitis B core antibody, hepatitis B e antigen, and hepatitis B e antibody using the electrochemiluminescence immuno-

Table 1 Background characteristics of 2304 patients with hepatocellular carcinoma *n* (%)	
Variables	***n* = 2304**
Sex	
Male	1987 (86.2)
Female	317 (13.8)
Age (yr), mean ± SD	51.9 ± 12.9
AFP value (μg/L), mean ± SD	555.31 ± 546.69
HBV infection	
No	429 (18.6)
Yes	1875 (81.4)
HCV infection	
No	2287 (99.3)
Yes	17 (0.7)
Child-Pugh grade	
A	1790 (77.7)
B	495 (21.5)
C	19 (0.8)

HCC: Hepatocellular carcinoma; AFP: α-fetoprotein; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

assay (ECLIA) method, and HBsAg positive was defined as HBV infection. Background characteristics of the patients are listed in Table 1.

AFP assay and assessment

Serum samples for the detection of AFP were taken upon entry into the study before initial treatment, and AFP was determined by ECLIA, which was intended for use on Roche MODULAR ANALYTICS E170 immunoassay analyzers (Roche Diagnostics GmbH, Mannheim, Germany). The time between collecting serum samples and performing an operation or getting a CT, US, MRI and/or angiography image was less than seven days. Detected serum AFP values ranged from 0 to 1210 μg/L, and all of the AFP values more than 1210 μg/L were recorded as 1210 μg/L in our study. The cutoff for normal AFP levels (< 20 μg/L) was chosen on the basis of the EASL guidelines[24] and on the data reported in the majority of previous studies[14,25]. Four different cutoff values for serum AFP were set: ≤ 20 μg/L, 21-400 μg/L, 401-800 μg/L, and ≥ 801 μg/L. Thus, patients were classified into four groups based on their level of serum AFP at the time of enrollment.

Clinicopathological variables

We assessed the association of AFP values with the clinicopathological variables that have been reported as prognostic factors for HCC[16,26]. The investigated variables are shown in Table 2. All variables were assessed either pathologically, *via* resected specimens and fine needle biopsy, or by imaging techniques. Vascular invasion was defined as the presence of portal vein invasion, venous invasion and/or biliary invasion. Tumor size was defined by the longest axis and estimated with US, CT or MRI. When multiple HCC tumors were present, the axis of the largest one was measured and taken as the representative HCC diameter. The diagnosis was histologically

Table 2 Serum α-fetoprotein level of different tumor related factors *n* (%)			
Variables	***n* = 2304**	**AFP (μg/L)**	***P* value**
HBV infection			< 0.001
Yes	1875 (81.4)	573.9 ± 547.7	
No	429 (18.6)	398.4 ± 522.3	
Tumor size(cm)			< 0.001
≤ 5	871 (37.8)	444.9 ± 514.2	
5-10	804 (34.9)	499.5 ± 536.4	
≥ 10	629 (27.3)	808.4 ± 529.2	
Tumor number			0.451
1	1666 (72.2)	531.9 ± 18.9	
2	288 (12.5)	532.2 ± 44.7	
≥ 3	350 (15.3)	556.0 ± 42.2	
Vascular invasion			< 0.001
No	1714 (74.4)	502.1 ± 543.1	
Yes	590 (25.6)	694.1 ± 546.9	
Tumor differentiation[1]			0.007
High	139 (7.5)	207.3 ± 420.8	
Intermediate[b]	963 (52.3)	527.9 ± 538.4	
Low	741 (40.2)	559.2 ± 545.7	
TNM staging			0.306
I	495 (21.5)	578.6 ± 549.3	
II	289 (12.5)	493.3 ± 542.2	
IIIA	726 (31.5)	548.6 ± 544.3	
IIIB	214 (9.3)	585.3 ± 553.4	
IIIC	101 (4.3)	558.9 ± 551.7	
IV	479 (20.8)	534.0 ± 545.9	
BCLC staging			0.008
A	869 (37.7)	506.2 ± 537.4	
B	251 (10.9)	590.1 ± 551.1	
C	1028 (44.6)	607.3 ± 553.3	
D[d]	156 (6.8)	625.7 ± 529.8	
China staging			0.386
I	137 (5.9)	487.8 ± 542.2	
IIa	198 (8.6)	528.8 ± 533.9	
IIb	893 (38.7)	560.8 ± 545.7	
III	1076 (46.7)	527.9 ± 544.8	

[1]We assessed 1843/2304 patients who had underwent hepatectomy or fine needle aspiration. [b]*P* < 0.001 *vs* high and low differentiation groups. [d]*P* < 0.001 *vs* stage A and B of BCLC. AFP: α-fetoprotein; HBV: Hepatitis B virus; TNM: Tumor-node-metastasis; BCLC: The Barcelona-Clinic Liver Cancer Group.

confirmed using resected specimens or fine needle aspiration biopsy, and the grading followed the methodology reported by Edmonson *et al*[27] (high, intermediate, or low differentiation). Similarly, if multiple tumors had more than two grades of histological differentiation, the most dedifferentiated grade was recorded.

Among the several tumor staging systems used in the world, the tumor-node-metastasis (TNM) system is the most widely accepted[28,29]. The American Joint Committee on Cancer (AJCC) published its 7th edition in 2009[30], and the main difference from the 6th edition of the AJCC staging system is the separation of the T3 stage into two subgroups, T3a and T3b. The definition of the T3a stage is the presence of multiple tumors, any > 5 cm, while the T3b stage is defined as having tumors of any size involving a major portal or hepatic vein. The tumor staging was also determined by the Barcelona Clinic Liver Cancer (BCLC) staging classification, which

Table 3 Positive rate of patients with different tumor-related factors in four α-fetoprotein level inervals *n* (%)

Variables AFP (μg/L)	≤ 20	20-400	401-800	≥ 801	*n* = 2304	*P* value
HBV infection						< 0.001
Yes	507 (26.7)	441 (23.2)	126 (6.4)	801 (42.4)	1875 (81.4)	
No	202 (47.0)	82 (19.2)	18 (4.3)	127 (29.5)	429 (18.6)	
Tumor size (cm)						< 0.001
≤ 5	243 (27.90)	361 (41.45)	66 (7.58)	201 (23.08)	871 (37.80)	
5-10	262 (32.59)	206 (25.62)	43 (5.35)	293 (36.44)	804 (34.90)	
≥ 10	117 (18.60)	84 (13.35)	27 (4.29)	401 (63.75)	629 (27.30)	
Tumor number						0.593
1	469 (28.15)	461 (27.67)	103 (6.18)	622 (38.00)	1666 (72.31)	
2	66 (22.92)	97 (33.68)	14 (4.86)	111 (38.54)	288 (12.50)	
≥ 3	87 (24.86)	93 (26.57)	19 (5.43)	151 (43.14)	350 (15.19)	
Vascular invasion						< 0.001
No	510 (29.75)	498 (29.05)	101 (5.89)	605 (35.30)	1714 (74.39)	
Yes	112 (19.98)	153 (25.93)	35 (5.93)	290 (49.15)	590 (25.61)	
Tumor differentiation						0.012
High	57 (42.22)	60 (44.44)	5 (3.70)	16 (11.85)	135 (5.99)	
Intermediate	285 (29.75)	263 (27.45)	81 (8.46)	335 (34.97)	958 (41.84)	
Low	263 (35.93)	134 (18.31)	43 (5.87)	301 (41.12)	732 (32.16)	
TNM staging						0.767
I	125 (25.25)	139 (28.08)	23 (4.65)	208 (42.02)	495 (21.48)	
II	90 (31.14)	81 (28.03)	18 (6.23)	100 (34.60)	289 (12.54)	
IIIA	197 (27.13)	201 (27.69)	50 (6.89)	278 (38.29)	726 (31.51)	
IIIB	50 (28.36)	64 (29.91)	10 (4.67)	90 (42.06)	214 (9.29)	
IIIC	27 (26.73)	28 (27.72)	5 (4.95)	41 (40.59)	101 (4.38)	
IV	133 (27.77)	138 (28.81)	30 (6.26)	178 (37.16)	479 (20.79)	
BCLC staging						< 0.001
A	261 (30.03)	266 (30.61)	56 (6.44)	286 (32.91)	869 (37.72)	
B	59 (23.51)	78 (31.08)	15 (5.98)	99 (39.44)	251 (10.89)	
C	271 (23.36)	272 (24.46)	53 (5.16)	432 (42.02)	1028 (44.62)	
D	31 (19.87)	35 (22.44)	12 (7.69)	78 (50.00)	156 (6.77)	
China staging						0.124
I	34 (24.82)	54 (39.42)	3 (2.19)	46 (33.58)	137 (5.95)	
IIa	48 (24.24)	70 (35.35)	7 (3.54)	73 (36.87)	198 (8.59)	
IIb	231 (25.87)	237 (26.54)	65 (7.28)	360 (40.31)	893 (38.76)	
III	309 (28.72)	290 (29.95)	61 (5.67)	416 (38.66)	1078 (46.70)	

Patients were divided into four groups according to the serum α-fetoprotein (AFP) level, they were ≤ 20, 20-400, 401-800 and ≥ 801 μg/L, the positive rate of patients in each interval of AFP were calculated. HBV: Hepatitis B virus; TNM: Tumor-node-metastasis; BCLC: The Barcelona-Clinic Liver Cancer Group.

was updated in 2010[31], and not involving the Cancer of the Liver Italian Program (CLIP) staging[32] because it includes AFP as a stage criteria. We also adopted the China Staging system, which was updated in 2001[33].

Statistical analysis

Values are presented as the mean ± SD. Correlations between markers values were analyzed by Spearman's rank correlation. Categorical binary variables were compared by χ^2. Associations between marker values and clinicopathological variables were analyzed by the Wilcoxon rank-sum test, and a binary logistic regression model for multiple variables analysis. *P* values of < 0.05 were accepted as statistically significant. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

The distribution of patients with respect to the etiology of the disease is reported in Table 1. Of the 2304 cases,

1987 patients were men and 317 patients were women (male-to-female ratio 6.27: 1). The mean serum level of AFP was 555.31 ± 546.69 μg/L. The mean patient age was 51.9 ± 12.9 years old. AFP levels were within the normal range (< 20 μg/L) in 27.4% (*n* = 631) of the cases. 81.4% (*n* = 1 875) patients were infected with HBV, and those patients had much higher AFP level (*P* < 0.001) compared with non-HBV infection ones.

AFP as a marker for clinicopathological variables representatives of tumor-related features

The correlation of AFP levels with tumor-related features is depicted in Tables 2, 3 and 4. The AFP level in patients with tumor sizes ≥ 10 cm was significantly higher (*P* < 0.001) than those patients with smaller tumors (tumor size < 10 cm). There was no significant difference between patients with one or multiple tumors (*P* = 0.451). AFP levels were remarkably higher in patients with vascular invasion, such that the AFP level was clearly higher than those levels in patients without evidence of vascular invasion (*P* < 0.001). Notably, there is also a

Table 4 Binary logistic regression analysis of tumor related factors elevating the level of α-fetoprotein (> 400 μg/L)

Variables	P value	Odds ratio (95%CI)
HBV infection	0.156	4.162 (0.991-16.557)
Vascular invasion	0.060	3.963 (0.529-7.384)
⩽ 5	-	-
5-10	0.341	0.862 (0.317-9.284)
⩾ 10	0.012	5.215 (1.426-13.151)
Tumor number		
1	-	-
2	0.513	0.487 (0.056-4.204)
⩾ 3	0.122	0.146 (0.013-1.671)
Tumor differentiation		
High	-	-
Intermediate	0.017	3.951 (1.501-9.917)
Low	0.006	6.362 (2.891-15.382)

The level status of α-fetoprotein (> 400 μg/L) was used as a dependent variable. HBV: Hepatitis B virus.

similar pattern with increasing tumor cell differentiation-patients with low tumor cell differentiation have the significantly highest AFP levels ($P = 0.007$). In the BCLC staging classification, AFP levels at various stages are significantly different ($P = 0.008$), with stage D having the highest level of AFP. No relationship or difference was found in the TNM staging and China staging classification systems.

Positive rates of patients with HCC in four AFP intervals

The positive rate of patients in four AFP intervals with and without HBV infection were quite different ($P < 0.001$), about half of the HBV infected patients with the AFP levels greater than 801 μg/L. On the contrary, only one fifth of the non-HBV infection patients had AFP levels more than 801 μg/L, AFP levels in half of the uninfected patients were less than 20 μg/L approximately. With respect to different tumor sizes, the positive rate of patient was significantly different ($P < 0.001$). The patients with the largest tumor size, ⩾ 10 cm, accounted for most of the cases in the interval where the AFP cutoff value was more than 801 μg/L (63.75%).Patients with tumor sizes either ⩽ 5 cm, 5-10 cm, or ⩾ 10 cm had the lowest rate of serum AFP in the AFP interval from 401 to 800 μg/L. The positive rate of serum AFP levels in different intervals was not significantly different in patients with solitary or multiple tumors ($P = 0.593$). Whether the patients with vascular invasion or not, the portion of AFP values beyond 801 μg/L accounted for the most cases (49.15% and 35.30% respectively), and the positive rate of AFP value was significantly different ($P < 0.001$). The high, intermediate, and low tumor cell differentiation had significant differences in each serum AFP interval. The low differentiation group accounted for the majority of patients in the AFP interval of more than 801 μg/L (41.12%), and the patients with high tumor cell differentiation had the highest negative rate (42.22%).The value of AFP had a statistically significant

correlation with BCLC, and the positive rate of the AFP level in the interval greater than 801 μg/L was found to be significantly different ($P < 0.001$). However, AFP levels greater than 801 μg/L tended to exist in the highest percentage of patients among all stages under the BCLC and China staging systems.

Multiple variables analysis of tumor related factors elevating the level of AFP

In the binary logistic multiple-regression models, the level status of AFP (> 400 μg/L) was used as a dependent variable. Both low tumor cell differentiation (OR 6.362, 95%CI: 2.891-15.382, $P = 0.006$) and tumor size (⩾ 10 cm) (OR 5.215, 95%CI: 1.426-13.151, $P = 0.012$) were independent predictors of elevated AFP concentrations.

DISCUSSION

Serum tumor markers are useful in detecting malignant carcinoma rapidly and simply using biochemical methods. In this study, we focused on AFP, which is the most commonly used tumor marker in HCC. To date, many previous studies have evaluated the relationship between serum AFP levels and tumor-related clinicopathological factors. However, in the majority of those studies, researchers[14,19,21,26] only took one or two clinicopathological variables into consideration, such as tumor size, tumor number, or portal venous invasion. Moreover, few of these researchers investigated these relationships in a comprehensive manner. With these facts in mind, we obtained a thorough understanding of the correlation between tumor markers and various clinicopathological variables.

It is known to all that, HBV is the major hepatocarcinogen which is responsible for up to 80% of HCC worldwide. There are over 400 million patients infected with chronic HBV globally, which equals to over 5% of the world's whole population; and it is estimated that about 20% of these infected individuals may eventually develop HCC[34]. Just as the relationship between HBV and HCC has been clarified, AFP is found higher in HBV-related HCC than in non-HBV-related HCC[35]. Hann *et al*[36] conducted a clinic based longitudinal cohort study (617 cases and followed for up to 22 years) retrospectively to determine the predictive role of baseline AFP value in the prediction of the long-term risk of developing HCC in HBV patients. Their conclusion was that elevated serum AFP was significantly associated with increased risk of HCC in HBV patients and that high levels of serum AFP were associated with the higher risk of developing HCC in non-cancer HBV patients. Our result indicated that 81.4% patients was in status of HBV infection and their serum AFP level raised up at quite a high level compared with non-HBV infection patient. Mutual influence of the HCC and HBV might have effect on the level of AFP.

Our study showed that patients' serum AFP concen-

trations were progressively higher with increasing tumor sizes, which is consistent with the results of previous studies[15-17], and the same pattern could be observed for the incidence rate of serum AFP greater than 801 μg/L. Specifically, the negative rates of serum AFP concentrations (< 20 μg/L) decreased as tumor sizes increased. This particular focus on tumor sizes and different cutoff values might be useful in suggesting criteria values for the screening and diagnosing of HCC. Moreover, it is still controversial as to whether AFP concentration has a positive correlation with tumor size worldwide[14,26,37]. One possible reason for this difference may be that the size of HCC tumors included in other studies was relatively smaller than in our study. We divided patients into three groups according to the longest axis: ⩽ 5 cm, 5-10 cm and ⩾ 10 cm. Nevertheless, the conventional division manner of tumor sizes in previous studies focused on a diameter ⩽ 5 cm. Another possible reason for these differences may be due to the difference in the tumor stages of the patients enrolled in these studies. As for the tumor sizes, the serum AFP level showed no tendency to increase with increasing tumor number, and the incidence of different intervals of the AFP level showed no clear correlation with tumor number as well. This result was not consistent with other studies[12], which showed a significant relationship between AFP levels and tumor number. The main difference was that the interindividual variation of secretion ability of tumor cell played a more important role in elevating the serum AFP concentration compared with tumor cell number.

The cause of cancer recurrence in HCC patients is mainly due to tumor cells spreading *via* the portal and hepatic veins. Additionally, vascular invasion has been proven to be an adverse prognostic factor for HCC recurrence. It is important to detect vascular invasion at earlier stages of the cancer. In our study, there was a relationship between AFP concentration and the presence of indices of tumor vascular invasiveness, that is, the serum AFP value was significantly higher in patients with various vascular invasions compared to those patients without vascular invasion. Among the patients with vascular invasion, the majority of them had AFP values greater than 801 μg/L (49.15%). Some early studies have shown that high serum AFP levels are a good indication of the higher incidence of vascular invasion in patients with HCC, but the total incidence of vascular invasion was 25.61% in all of our cases, which is slightly lower than some other studies[21,38,39]. This deviation from the literature might be due to the definition of vascular invasion in our study, which focused on the involvement of the vessels within the portal and hepatic vein and biliary ducts, not including the vessels within the fibrous tumor capsule.

Before patients underwent hepatectomy, it is impossible to exactly distinguish the degree of differentiation of the tumor cell by imaging techniques, and fine needle aspiration is not recommended because it has the risk of the cancer seeding. Hence, a serum AFP value greater than 400 μg/L might be a useful criterion for predicting the histological tumor grade[19]. In the present study, the serum AFP value increased according to the change of tumor cell differentiation from high to low differentiation, which strongly suggests that the tumor cell with elevated malignant potential will relatively enhance the secretion capacity. Previous studies that investigated the relationship between AFP and tumor cell differentiation, namely Kentaroh Yamamoto[14], Koicii Oishie[19] and Chaur-Shine Wang[16] and Fabio Farinati[40], reached the same conclusion. Together, these findings highlighted that during the course of the back and forth liver cell necrosis and regeneration, cell proliferation and serum AFP values are abnormally elevated. With respect to the tumor staging system, several staging systems are available, such as Okuda, CLIP, BCLC, Child-Pugh-staging, TNM staging and the French classification. In terms of our study, the main function of tumor staging is presented in three main concepts: the malignant characteristics of tumor cells, the degree of liver impairment and the patients' general condition. As a result, BCLC and TNM staging are used in our study. Another staging system that was included was China staging. With our data, BCLC had the dominant advantage compared to the other two staging systems given that it showed a clear relationship with increasing AFP levels. This result may be due to BCLC integrating the Okuda and Child-Pugh staging systems, which consist of tumor features and conditions of liver functions. The serum AFP level of 400 μg/L was adopted as the cutoff value based on the Milan and Hangzhou criterion[41] for liver transplantation. In our study, patients with AFP levels less than 400 μg/L had less vascular invasion and were at a lower tumor stage. Based on Hangzhou criteria, HCC patients with a simultaneous total tumor diameter of smaller than 8 cm, histopathology grade of I or II and a preoperative AFP level of less than or equal to 400 μg/L, can be selected for liver transplantation and have good outcomes.

The limitation of this study is that it does not include other serum tumor markers, such as AFP-L3 and desgamma-carboxy prothrombin (DCP), to assess the correlation among them and to compare differences in their relationship with various clinicopathological variables. AFP-L3 and DCP have been proposed as complements or substitutes in the diagnosis of HCC. A high serum AFP-L3 fraction has been proven to be closely related to the poor differentiation of HCC[42]. Research has also indicated that a high serum DCP is more useful than AFP in the differential diagnosis of HCC from other benign hematopathologist and is unique in detecting small tumors in patients[43]. The reason we did not include AFP-L3 and DCP is that few cases have examined these markers in our hospital so far. Next, we plan to accumulate enough HCC cases with DCP and AFP-L3 data to investigate the detailed relationship with HCC and to compare them to the relationship with the serum AFP

concentration.

Although some clinical doctors regard serum AFP as 'obsolete', measuring AFP concentrations in the blood is still the easiest and preferred serum tumor marker for the current screening and diagnosing of HCC in China. Despite the promising results of these newly discovered serum tumor markers, such as DCP and AFP-L3, they are only regarded as a complementary test to the AFP value and currently cannot match the diagnostic and prognostic value of AFP. At least for the proportion of AFP seropositive patients, AFP is indeed demonstrated to be associated with larger tumor sizes, vascular invasion and low tumor differentiated grades, which confirmed the rationality of using BCLC with AFP values in our study. However, physicians should be aware that neither a positive nor a negative serum AFP result is conclusive for the final diagnosis of HCC.

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COMMENTS

Background
Hepatocellular carcinoma (HCC) is one of the most common and important cancers in the world that is associated with a poor prognosis. Although imaging studies are the most important and valuable diagnostic tool for HCC, α-fetoprotein (AFP) is also indispensable for diagnosing HCC. Regarding the clinicopathological features of HCC with AFP, several investigators have reported a high frequency of vascular invasions, intrahepatic metastasis, large tumor size, low differentiation, multiple tumor number, and worse survival in HCC patients with high levels of AFP.

Research frontiers
AFP has been the most widely used tumor marker worldwide and is still the golden standard amongst diagnostic markers for HCC. However, its diagnostic value is more and more questioned, due to poor sensitivity and specificity. To date, several other tumor markers have been investigated and compared with AFP, such as plasma des-c-carboxy prothrombin, also known as protein induced by vitamin K deficiency or antagonist-II, the lens culinaris agglutinin-reactive fraction of α-fetoprotein and Golgi protein-73 have been investigated as complements for AFP, despite the promising results of these new potential markers, at this moment, they are only recommended as complementary tests to the conventionally diagnostic methods used and cannot (yet) replace serum AFP as the gold standard of tumour markers for HCC. As a result, a comprehensive investigation with large samples in regard to the relationship between clinicopathological features of HCC and AFP is needed urgently.

Innovations and breakthroughs
The authors analyzed the correlation between serum AFP levels and characteristics of the patients with HCC. Together with the sample size of the study, which is large enough to obtain statistically relevant results, this article could be useful for the physicians at the clinical practice, keeping in mind that represent a guidelines for the clinicopathological features of HCC, as long as the AFP result is accompanied by other diagnosis techniques.

Applications
The present study provides a whole description of the relationship of various clinicopathological features of HCC with serum level of AFP, these finding in the article will play an important role in clinical decision making in future.

Terminology
AFP is a glycoprotein and synthesized by the yolk sac during early foetal life and later on by the foetal liver. In adult life, AFP synthesis is repressed. Elevated serum levels of AFP are only seen in maternal serum during pregnancy, in certain tumors (gastric cancer, lung cancer, pancreatic cancer, testicular carcinoma and mainly in HCC) and hepatic non-tumor disease (chronic hepatitis and liver cirrhosis).

Peer review
This is a large study about the diagnostic and prognostic abilities of AFP in hepatocellular carcinoma. The series is impressive and various interesting conclusions are reached. In this sense, the article has merit and would represent a valuable contribution to the literature.

REFERENCES

1   **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750 DOI: 10.1016/S0140-6736(03)14964-1]

2   **Parkin DM**, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; **94**: 153-156 [PMID: 11668491 DOI: 10.1002/ijc.1440]

3   **El-Serag HB**, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; **340**: 745-750 [PMID: 10072408 DOI: 10.1056/NEJM199903113401001]

4   **Collier J**, Sherman M. Screening for hepatocellular carcinoma. *Hepatology* 1998; **27**: 273-278 [PMID: 9425947 DOI: 10.1002/hep.510270140]

5   **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]

6   **Wu W**, Yao DF, Yuan YM, Fan JW, Lu XF, Li XH, Qiu LW, Zong L, Wu XH. Combined serum hepatoma-specific alpha-fetoprotein and circulating alpha-fetoprotein-mRNA in diagnosis of hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 538-544 [PMID: 17085339 DOI: CNKI:]

7   **Marubashi S**, Nagano H, Wada H, Kobayashi S, Eguchi H, Takeda Y, Tanemura M, Umeshita K, Doki Y, Mori M. Clinical significance of alpha-fetoprotein mRNA in peripheral blood in liver resection for hepatocellular carcinoma. *Ann Surg Oncol* 2011; **18**: 2200-2209 [PMID: 21301972 DOI: 10.1245/s10434-011-1577-7]

8   **Ijichi M**, Takayama T, Matsumura M, Shiratori Y, Omata M, Makuuchi M. alpha-Fetoprotein mRNA in the circulation as a predictor of postsurgical recurrence of hepatocellular carcinoma: a prospective study. *Hepatology* 2002; **35**: 853-860 [PMID: 11915031 DOI: 10.1053/jhep.2002.32100]

9   **Sun GZ**, Zhao XY, Li JH, Zhao GQ, Wang SX, Kong SL. [Detection of alpha-fetoprotein-L3 using agglutinin-coupled spin column to be used in diagnosis of hepatocellular carcinoma]. *Zhonghua Yi Xue Zazhi* 2008; **88**: 1986-1988 [PMID: 19062741]

10   **Sassa T**, Kumada T, Nakano S, Uematsu T. Clinical utility of simultaneous measurement of serum high-sensitivity des-gamma-carboxy prothrombin and Lens culinaris agglutinin A-reactive alpha-fetoprotein in patients with small hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 1999; **11**: 1387-1392 [PMID: 10654799 DOI: 10.1097/00042737-199912000-00008]

11   **Ang IL**, Poon TC, Lai PB, Chan AT, Ngai SM, Hui AY, Johnson PJ, Sung JJ. Study of serum haptoglobin and its glycoforms in the diagnosis of hepatocellular carcinoma: a glycoproteomic approach. *J Proteome Res* 2006; **5**: 2691-2700 [PMID: 17022640 DOI: 10.1021/pr060109r]

12   **Carr BI**, Kanke F, Wise M, Satomura S. Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. *Dig Dis Sci* 2007; **52**: 776-782 [PMID: 17253135 DOI: 10.1007/

s10620-006-9541-2]

13   **Okuda H**, Nakanishi T, Takatsu K, Saito A, Hayashi N, Takasaki K, Takenami K, Yamamoto M, Nakano M. Serum levels of des-gamma-carboxy prothrombin measured using the revised enzyme immunoassay kit with increased sensitivity in relation to clinicopathologic features of solitary hepatocellular carcinoma. *Cancer* 2000; **88**: 544-549 [PMID: 10649245 DOI: 10.1002/(SICI)1097-0142(20000201)88]

14   **Yamamoto K**, Imamura H, Matsuyama Y, Hasegawa K, Beck Y, Sugawara Y, Makuuchi M, Kokudo N. Significance of alpha-fetoprotein and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma undergoing hepatectomy. *Ann Surg Oncol* 2009; **16**: 2795-2804 [PMID: 19669841 DOI: 10.1245/s10434-009-0618-y]

15   **Nakamura S**, Nouso K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, Toshikuni N, Tanaka H, Miyake Y, Matsumoto E, Shiratori Y. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *Am J Gastroenterol* 2006; **101**: 2038-2043 [PMID: 16848811 DOI: 10.1111/j.1572-0241.2006.00681.x]

16   **Wang CS**, Lin CL, Lee HC, Chen KY, Chiang MF, Chen HS, Lin TJ, Liao LY. Usefulness of serum des-gamma-carboxy prothrombin in detection of hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 6115-6119 [PMID: 16273636]

17   **Kasahara A**, Hayashi N, Fusamoto H, Kawada Y, Imai Y, Yamamoto H, Hayashi E, Ogihara T, Kamada T. Clinical evaluation of plasma des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma in patients with tumors of various sizes. *Dig Dis Sci* 1993; **38**: 2170-2176 [PMID: 7505217 DOI: 10.1007/BF01299891]

18   **Saffroy R**, Pham P, Reffas M, Takka M, Lemoine A, Debuire B. New perspectives and strategy research biomarkers for hepatocellular carcinoma. *Clin Chem Lab Med* 2007; **45**: 1169-1179 [PMID: 17635075 DOI: 10.1515/cclm.2007.262]

19   **Oishi K**, Itamoto T, Amano H, Fukuda S, Ohdan H, Tashiro H, Shimamoto F, Asahara T. Clinicopathologic features of poorly differentiated hepatocellular carcinoma. *J Surg Oncol* 2007; **95**: 311-316 [PMID: 17326126 DOI: 10.1002/jso.20661]

20   **Mitsuhashi N**, Kobayashi S, Doki T, Kimura F, Shimizu H, Yoshidome H, Ohtsuka M, Kato A, Yoshitomi H, Nozawa S, Furukawa K, Takeuchi D, Suda K, Miura S, Miyazaki M. Clinical significance of alpha-fetoprotein: involvement in proliferation, angiogenesis, and apoptosis of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2008; **23**: e189-e197 [PMID: 18466288 DOI: 10.1111/j.1440-1746.2008.05340.x]

21   **Sakata J**, Shirai Y, Wakai T, Kaneko K, Nagahashi M, Hatakeyama K. Preoperative predictors of vascular invasion in hepatocellular carcinoma. *Eur J Surg Oncol* 2008; **34**: 900-905 [PMID: 18343084 DOI: 10.1016/j.ejso.2008.01.031]

22   **Han K**, Tzimas GN, Barkun JS, Metrakos P, Tchervenkov JL, Hilzenrat N, Wong P, Deschênes M. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. *Can J Gastroenterol* 2007; **21**: 39-45 [PMID: 17225881]

23   **Talwalkar JA**, Gores GJ. Diagnosis and staging of hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S126-S132 [PMID: 15508076 DOI: 10.1053/j.gastro.2004.09.026]

24   **European Association for Study of Liver; European Organisation for Research and Treatment of Cancer**. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *Eur J Cancer* 2012; **48**: 599-641 [PMID: 22424278 DOI: 10.1016/j.ejca.2011.12.021]

25   **Yamamoto K**, Imamura H, Matsuyama Y, Kume Y, Ikeda H, Norman GL, Shums Z, Aoki T, Hasegawa K, Beck Y, Sugawara Y, Kokudo N. AFP, AFP-L3, DCP, and GP73 as markers for monitoring treatment response and recurrence and as surrogate markers of clinicopathological variables of HCC. *J Gastroenterol* 2010; **45**: 1272-1282 [PMID: 20625772

DOI: 10.1007/s00535-010-0278-5]

26   **Lu XY**, Xi T, Lau WY, Dong H, Xian ZH, Yu H, Zhu Z, Shen F, Wu MC, Cong WM. Pathobiological features of small hepatocellular carcinoma: correlation between tumor size and biological behavior. *J Cancer Res Clin Oncol* 2011; **137**: 567-575 [PMID: 20508947 DOI: 10.1007/s00432-010-0909-5]

27   **Edmondson HA**, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer* 1954; **7**: 462-503 [PMID: 13160935 DOI: 10.1002/1097-0142(195405)7]

28   **Henderson JM**, Sherman M, Tavill A, Abecassis M, Chejfec G, Gramlich T. AHPBA/AJCC consensus conference on staging of hepatocellular carcinoma: consensus statement. *HPB (Oxford)* 2003; **5**: 243-250 [PMID: 18332995 DOI: 10.1080/13651820310015833]

29   **Lu W**, Dong J, Huang Z, Guo D, Liu Y, Shi S. Comparison of four current staging systems for Chinese patients with hepatocellular carcinoma undergoing curative resection: Okuda, CLIP, TNM and CUPI. *J Gastroenterol Hepatol* 2008; **23**: 1874-1878 [PMID: 18752560 DOI: 10.1111/j.1440-1746.2008.05527.x]

30   **Edge SB**, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17**: 1471-1474 [PMID: 20180029 DOI: 10.1245/s10434-010-0985-4]

31   **Forner A**, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis* 2010; **30**: 61-74 [PMID: 20175034 DOI: 10.1055/s-0030-1247133]

32   A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* 1998; **28**: 751-755 [PMID: 9731568 DOI: 10.1002/hep.510280322]

33   **Yang BH**, Xia JL. Clinical staging and diagnosis criteria for primary liver carcinoma in China. *Zhongguo Ganzangbing Zazhi* 2001; **9**: 324

34   **Blum HE**, Moradpour D. Viral pathogenesis of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2002; **17** Suppl 3: S413-S420 [PMID: 12472973 DOI: 10.1046/j.1440-1746.17.s3.37.x]

35   **Kim HS**, Park JW, Jang JS, Kim HJ, Shin WG, Kim KH, Lee JH, Kim HY, Jang MK. Prognostic values of alpha-fetoprotein and protein induced by vitamin K absence or antagonist-II in hepatitis B virus-related hepatocellular carcinoma: a prospective study. *J Clin Gastroenterol* 2009; **43**: 482-488 [PMID: 19197197 DOI: 10.1097/MCG.0b013e318182015a]

36   **Hann HW**, Fu X, Myers RE, Hann RS, Wan S, Kim SH, Au N, Xing J, Yang H. Predictive value of alpha-fetoprotein in the long-term risk of developing hepatocellular carcinoma in patients with hepatitis B virus infection--results from a clinic-based longitudinal cohort. *Eur J Cancer* 2012; **48**: 2319-2327 [PMID: 22436980 DOI: 10.1016/j.ejca.2012.02.065]

37   **Johnson PJ**. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. *Clin Liver Dis* 2001; **5**: 145-159 [PMID: 11218912 DOI: 10.1016/S1089-3261(05)70158-6]

38   **Ibrahim S**, Roychowdhury A, Hean TK. Risk factors for microvascular tumour thrombi in hepatocellular carcinoma: a univariate and multivariate analysis. *ANZ J Surg* 2007; **77**: 146-149 [PMID: 17305988 DOI: 10.1111/j.1445-2197.2006.03995.x]

39   **Tsai TJ**, Chau GY, Lui WY, Tsay SH, King KL, Loong CC, Hsia CY, Wu CW. Clinical significance of microscopic tumor venous invasion in patients with resectable hepatocellular carcinoma. *Surgery* 2000; **127**: 603-608 [PMID: 10840353 DOI: 10.1067/msy.2000.105498]

40   **Farinati F**, Marino D, De Giorgio M, Baldan A, Cantarini M, Cursaro C, Rapaccini G, Del Poggio P, Di Nolfo MA, Benvegnù L, Zoli M, Borzio F, Bernardi M, Trevisani F. Diagnostic and prognostic role of alpha-fetoprotein in

hepatocellular carcinoma: both or neither? *Am J Gastroenterol* 2006; **101**: 524-532 [PMID: 16542289 DOI: 10.1111/j.1572-0241.2006.00443.x]

41   **Zheng SS**, Xu X, Wu J, Chen J, Wang WL, Zhang M, Liang TB, Wu LM. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008; **85**: 1726-1732 [PMID: 18580463 DOI: 10.1097/TP.0b013e31816b67e4]

42   **Oka H**, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, Osaki Y, Seki T, Kudo M, Tanaka M. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein. *J Gastroenterol Hepatol* 2001; **16**: 1378-1383 [PMID: 11851836 DOI: 10.1046/j.1440-1746.2001.02643.x]

43   **Fujioka M**, Nakashima Y, Nakashima O, Kojiro M. Immunohistologic study on the expressions of alpha-fetoprotein and protein induced by vitamin K absence or antagonist II in surgically resected small hepatocellular carcinoma. *Hepatology* 2001; **34**: 1128-1134 [PMID: 11732002 DOI: 10.1053/jhep.2001.29202]