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Original Article

Elevated AFP, ATV, and AFP/ATV ratios as risk factors for poor prognosis of HCC patients in the BCLC-A stage

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Abstract: Objective: The aim of the current study was to evaluate the predictive value of alpha-fetoprotein (AFP), actual tumor volume (ATV), and AFP/ATV ratios for post-operation recurrence and poor prognosis in hepatocellular carcinoma (HCC) patients. Methods: This retrospective study enrolled 302 patients with HCC at the BCLC-A stage, between January 2007 and December 2014. They were divided into subgroups based on pre-op AFP levels, ATV sizes, and AFP/ATV ratios. Correlation levels between these parameters and post-operation disease-free survival (DFS) rates were analyzed. Results: Multivariate analysis revealed that pre-op AFP, ALT, intact tumor capsule, and ATV were independent factors for poor prognosis of HCC patients in the BCLC-A stage. AFP-positive patients had significantly lower 1-year, 3-year, and 5-year DFS rates ($P < 0.05$ compared to AFP-negative cases). Patients with higher pre-op AFPs also had lower DFS rates than those with lower AFP levels. Pre-operation ATV was negatively correlated with 1-year, 3-year, and 5-year DFS rates. AFP/ATV ratios were also negatively associated with DFS values. Sensitivity and specificity levels were 60.8% and 79.1%, respectively, when the cut-off value was 21.72. Conclusion: Pre-op AFP/ATV ratios are crucial in predicting post-op recurrence risks for HCC patients in the BCLC-A stage.

Keywords: Hepatocellular carcinoma, radical tumor resection, pre-op AFP and ATV, AFP/ATV, post-op prognosis

Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer morbidity and mortality in many countries around the world, especially in Asia and sub-Saharan Africa [1]. China is an epidemic region for HCC, with more than 200,000 deaths occurring annually [2], accounting for more than half of the patients worldwide [3]. At present, alpha-fetoprotein (AFP) and hepatic ultrasounds are conventional methods for HCC screening. However, sensitivity and specificity levels of these methods are not satisfactory. Moreover, the extent of AFP elevation is not entirely correlated with tumor size. Therefore, it is necessary to identify a reliable predictive index for prognosis of HCC.

Although actual tumor volume (ATC) is a novel predictive index for prognosis of HCC patients and has a relatively higher reliability in evaluating tumor load, it is still biased due to irregular growth patterns of tumors. The combination of ATV and AFP has obtained satisfactory results in prognostic evaluations of liver transplantation. These may be the ideal choice [4]. However, this index has not been used in evaluating the prognosis of HCC patients undergoing radical resections. Thus, the current retrospective study was conducted, aiming to assess the predictive value of pre-op levels of AFP, ATV, and AFP/ATV ratios for prognosis of HCC patients.

Materials and methods

Patients

The current retrospective study enrolled all HCC patients in the BCLC-A stage that received radical resections of tumors, from January 2007 to December 2014. Inclusion criteria: 1) HCC patients in the BCLC-A stage; 2) Receiving HCC radical resections; 3) Confirmed HCC via pathology; 4) Elevated pre-op AFP declined to normal levels within 2 months after surgery; and 5) With complete medical history data. Exclusion

criteria: 1) Patients receiving chemotherapy, intervention, or radiotherapy before surgery; 2) Complicated with distal metastasis; 3) Recurrence within 2 months after surgery; and 4) Patients with abnormal AFP levels at 2 months post-operation.

Criteria and groups

Serum AFP was quantified using the radioimmunology method. Pre-op negative AFP was defined as less than 20 ng/mL. Patients were divided into AFP-positive (higher than 20 ng/mL) and AFP-negative groups. Post-operation recurrence was identified [5] when: 1) Imaging showed *de novo* lesions or extra-hepatic metastatic lesions; 2) No decreases or persistently high AFP levels; and 3) Decreased AFP after surgery, but soon increased again. ATV was calculated using the formula: ATV=π X (4/3) * a * b * c. In this equation, a, b, and c represent 3-dimensional diameters of tumor lesions, π=3.14.

Post-operation follow-ups

Patients were followed up via telephone interviews or out-patient clinic visits after surgery. Follow-ups were conducted in each month within the first half year after surgery, every three months during the second half-year, and every six months after 2 years. All follow-ups ended on December 30, 2014. Survival time was defined as the period from surgery day to recurrence, death, or the last follow-up. Various information was recorded, including liver function assays, abdominal ultrasounds, CT results, and MRI results.

Statistical methods

SPSS 19.0 software (SPSS, Chicago, IL, USA) was used for data analysis. Categorical variables are expressed as percentages. Chi-square tests or Fisher's exact tests were used for comparisons. Cox's regression model was used to analyze risk factors affecting prognosis. Receiver operating characteristic (ROC) curves were applied to predict the cut-off value of ATC. The cut-off point was confirmed using the Youden index, defined as (sensitivity + specificity)-1. Survival analysis was performed via the Kaplan-Meier method or lifespan table approach. This was followed by log-rank or Gehan testing. Pearson's analysis was used to analyze the relationship between pre-op AFP and pathological differentiation types, ATV and pathological differentiation levels, and pre-op AFP and ATV levels. Statistical significance is defined as $P < 0.05$.

Results

A total of 302 patients were finally recruited, according to inclusion criteria. All patients received radical tumor resections. A total of 96 had regular resections, while 206 cases received irregular resections. There were 147 AFP-positive (higher than 20 ng/mL) individuals plus 155 AFP-negative patients. The median length of follow-up was 3.5 years. Detailed medical records of all patients are shown in **Table 1**.

Cox's regression analysis

Cox's regression model was established to analyze prognostic factors for all 302 patients (**Table 2**). Single-variate analysis revealed pre-op AFP, pre-op ALT, the integrity of tumor capsule, age, sex, and ATV as prognostic factors affecting HCC patient prognosis ($P < 0.05$). Multivariant analysis also revealed pre-op AFP, pre-op ALT, the integrity of tumor capsule, and ATV as independent factors affecting the prognosis of HCC patients in the BCLC-A stage ($P < 0.05$).

In the 302 HCC patients in the BCLC-A stage, 1-year, 3-year, and 5-year DFS rates were 82%, 66%, and 58%, respectively. These DFS rates in AFP-negative individuals were 85%, 71%, and 63%, respectively. They were 79%, 60%, and 51% in AFP-positive ones. Therefore, AFP-positive patients had lower survival rates than the AFP-negative group (χ^2=5.545, P=0.019, **Table 3**; **Figure 1A**).

Pre-op AFP positive patients were further divided into three groups, including 20-200 ng/mL, 200-400 ng/mL, and ≥ 400 ng/mL groups. The 1-year, 3-year and 5-year DFS rates were 82.8%, 74.4%, and 67.7% for the 20-200 ng/mL AFP group, 78.4%, 63.7%, and 55.7% for the 200-400 ng/mL AFP group, and 73.1%, 52.9%, and 36.6% for the ≥ 400 ng/mL AFP group. Therefore, HCC patients with higher AFP (≥ 400 ng/mL) had lower DFS rates than moderate (200-400 ng/mL) (χ^2=6.338, P=0.012) and low (20-200 ng/mL) AFP ones (χ^2=4.536,

Table 1. Comparison of clinical information between AFP-positive and AFP-negative HCC patients

Clinical index	BCLC-A stage HCC		X^2	*P*
	AFP-positive	AFP-negative		
Sex				
Male	130	140	0.284	0.594
Female	17	15		
Age				
≥ 60 years	20	38		
40~60 years	96	93	7.103	0.029
≤ 40 years	34	24		
Pre-op ALT				
≥ 40 U/L	72	52	6.690	0.082
< 40 U/L	75	95		
Pre-op AST				
≥ 40 U/L	57	45	3.937	0.268
< 40 U/L	90	102		
Maximal tumor size				
< 3 cm	36	24		
3 ≤ & < 5	47	54	1.083	0.582
5 ≤ & < 10	51	64		
≥ 10	13	13		
Integrity of tumor capsule				
Intact	90	116	7.117	0.028
Injured	57	39		
Resection edge				
≥ 1 cm	83	76	1.844	0.175
< 1 cm	64	79		
Ethanol injection				
Yes	48	56	0.341	0.559
No	99	99		
Liver cirrhosis				
Yes	132	121	1.724	0.442
No	15	34		
Regular resection				
Yes	48	48	0.126	0.772
No	99	107		
Pathology subtype				
Low differentiation	57	24		
Moderate differentiation	82	57	70.901	< 0.001
High differentiation	8	74		

P=0.033). No significant differences in DFS rates existed between moderate and high AFP groups (χ^2=0.559, *P*=0.455, **Table 3**; **Figure 1B**). Correlation analysis revealed a slight correlation between AFP and pathology differentiation types (P=0.006, r=0.224, **Table 4**).

Correlation between ATV and post-op DFS rates

A ROC curve was plotted to describe the correlation between ATV and post-op recurrence (**Figure 1C**). The area-under-curve (AUC) was 0.671 (95% *CI*, 0.610-0.732, *P* < 0.01), with 35.4% sensitivity and 90.9% specificity levels. ATV=13.78 cm^3 can work as the best predictive cut-off value for post-op recurrence of HCC patients in the BCLC-A stage.

Thus, the patients were further divided into ATV < 13.78 cm^3 and ATV ≥ 13.78 cm^3. The former group had an 86% 1-year DFS rate, 74% 3-year DFS rate, and 63% 5-year DFS rate. The latter group had a 79% 1-year DFS, 50% 3-year DFS, and 36% 5-year DFS, with statistically significant differences (χ^2=15.135, P < 0.001, **Table 3**; **Figure 1D**). Correlation analysis showed no relationship between ATV and pathology differentiation types (r=0.081, P=0.161, **Table 4**).

Correlation between pre-op AFP or AFP/ATV ratios and post-op DFS rates

A ROC curve was plotted to describe the correlation between post-op recurrence and AFP/ATV (**Figure 1E**). The area under curve was 0.721 (95% *CI*, 0.662-0.779, *P* < 0.001), with 60.8% sensitivity and 79.1% specificity levels. AFP/ATV=21.72 works as the best predictive value for judging post-op recurrence of HCC patients in the BCLC-A stage.

The HCC patients were divided into AFP/ATV < 21.71 and AFP/ATV ≥ 21.72. The former group had an 89% 1-year DFS rate, 79% 3-year DFS rate, and 69% 5-year DFS rate. The latter group had a 78% 1-year DFS, 53% 3-year DFS, and 35% 5-year DFS, with statistically significant differences (χ^2=65.374, *P* < 0.001, **Table 3**; **Figure 1F**).

Table 2. Single-variable and multi-variable analysis of 302 HCC patients

Prognostic factor	Single variate		Multi-variate	
	HR	*P*-value	HR	*P*-value
Maximal tumor size	1.021	0.476	-	-
Pre-op AFP	1.645	0.006	1.645	0.006
Pre-op ALT	1.005	0.016	1.005	0.016
Pre-op AST	1.005	0.228	-	-
Integrity of tumor capsule	0.570	0.007	0.570	0.007
Liver cirrhosis	0.751	0.227	-	-
Age	1.442	0.048	1.450	0.053
Cutting edge distance	0.760	0.136	-	-
Surgical route	1.136	0.511	-	-
Absolute ethanol	1.179	0.388	-	-
Sex	0.436	0.033	0.631	0.219
ATV	2.448	0.000	-	-
Pathology type	0.867	0.229	2.448	< 0.001

Table 3. Comparison of DFS rates between AFP-negative and AFP-positive patients

Group	N	DFS rate (%)		
		1-year	3-year	5-year
AFP				
All	302	82	66	58
AFP-negative	155	85	71	63
AFP-positive	147	79	60	51
20-200 ng/ml	29	82.8	74.4	67.6
200-400 ng/ml	51	78.4	63.7	55.7
≥ 400 ng/ml	67	73.1	52.9	36.6
ATV				
< 13.78 cm^3	241	86	74	63
≥ 13.78 cm^3	61	79	50	36
AFP/ATV				
< 21.72	190	89	79	69
≥ 21.72	112	78	53	35

Correlation analysis showed a weak correlation between ATV and pre-op AFP levels (r=0.036, P=0.1121, **Table 5**).

Discussion

AFP is an HCC-related marker with significant specificity. However, certain non-HCC diseases, such as chronic hepatitis or pregnancies, may also present elevated AFP levels, while certain HCC patients have negative AFP expression. Moreover, the degree of AFP elevation may not be parallel with tumor size. These factors compromise sensitivity or specificity levels of AFP in HCC diagnosis. Therefore, the sole dependence on AFP for prognostic prediction of HCC needs to be optimized [6]. The current study found that pre-op AFP, ALT, intact tumor capsule, and ATV are all independent risk factors for poor prognosis of HCC patients. Patients with ≥ 400 ng/mL AFP, ≥ 13.78 cm^3 ATV, and ≥ 21.72 AFP/ATV ratios have a relatively worse prognosis. Pre-op AFP/ATV ratios, therefore, may work as a crucial index predicting post-op recurrence for HCC patients in the BCLC-A stage.

About 50%~70% of HCC patients have higher than normal levels of serum AFP. Thus, AFP levels have been widely used for HCC diagnosis, efficiency evaluation, and prediction of recurrence and prognosis, with 40%~65% sensitivity and 76%~96% specificity. At present, most studies agree that elevated AFP is a high-risk factor for tumor recurrence. Either sensitivity or specificity levels of AFP elevation are not satisfactory in predicting tumor prognosis. In this study, HCC patients with positive pre-op AFPs had lower post-op DFS rates than the AFP-negative group, indicating a correlation between AFP positive expression and unfavorable prognosis in HCC patients in the BCLC-A stage. Present results are in accord with those of Ma et al. [7]. Further, stratified analysis showed the lowest post-op DFS rates in AFP ≥ 400 ng/mL patients, compared to those with 20-200 ng/mL or 200-400 ng/mL AFP, in accord with previous reports [8, 9]. Three major reasons might explain the unfavorable prognosis of HCC patients in the BCLC-A stage with pre-op AFP ≥ 400 ng/mL. These include immune suppression, pathology differentiation, and activity of peritumor cell mass. First, human immune status is closely correlated with tumor occurrence and progression. AFP significantly inhibits the antigen presenting potency of DC cells and decreased NK cell activity, impeding cellular body immunity [10]. Immune function of HCC patients, however, was not systemically evaluated, due to the retrospective nature of this study. Second, previous studies have claimed that AFP upregulation facilitates pathology differentiation process of HCC [11, 12]. Correlation analysis, in the current study, showed a positive relationship between AFP and pathology differentiation types. In all AFP-positive patients, more than 90% belonged to the moderate or

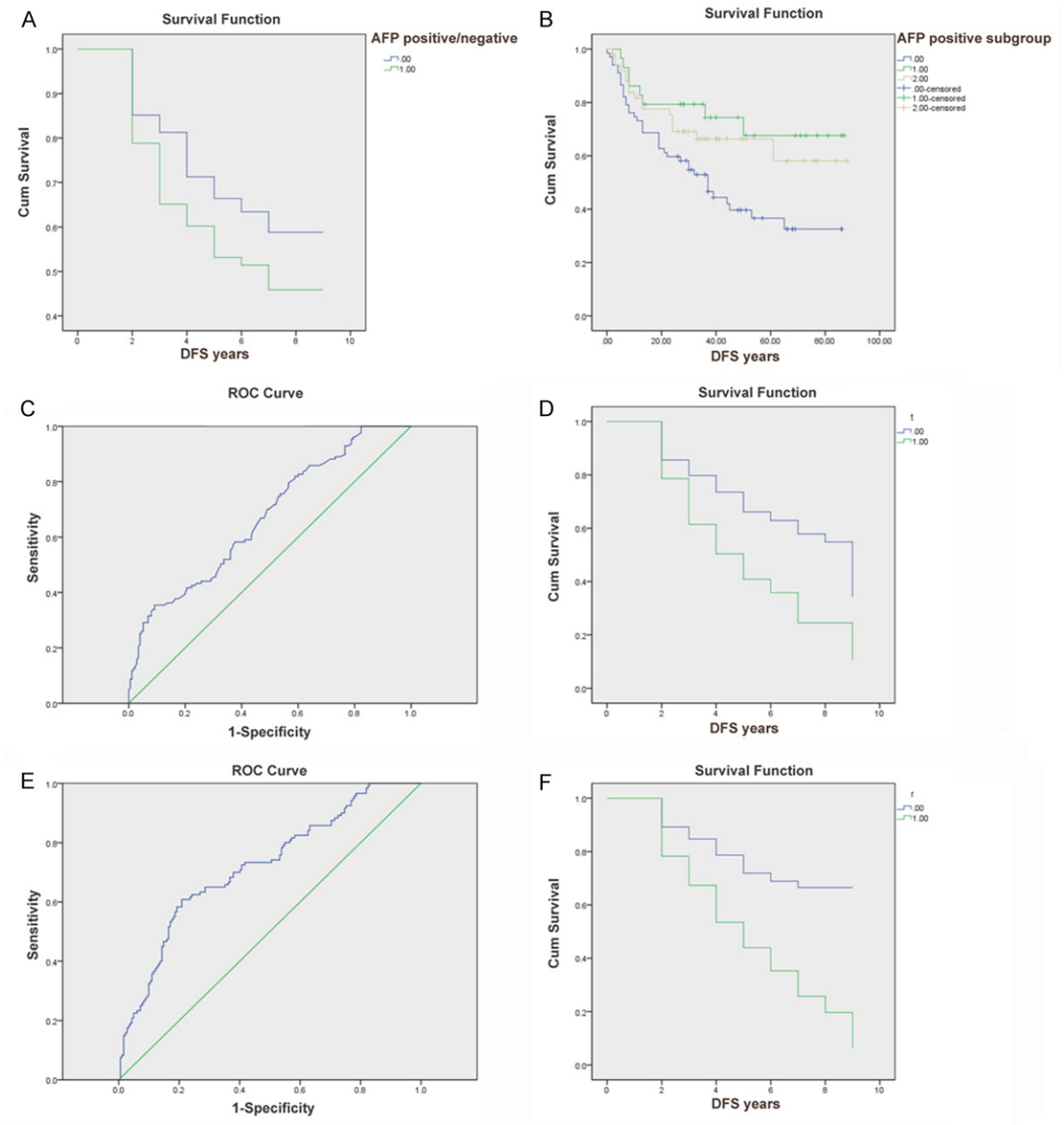

Figure 1. Kaplan-Meier survival analysis and Receiver Operating Characteristic (ROC) curves. A: Disease-free survival rates between AFP-negative and AFP-positive patients; 0.00, negative group; 1.00 positive group; B: Post-op DFS rates and AFP levels in HCC patients; 0.00, ≥ 400 ng/ml AFP group; 1.00, 20-200 ng/mL AFP group; 2.00, 200-400 ng/mL AFP group; C: ROC curve for ATV and post-op recurrence; D: Correlation between ATV and post-op DFS rates; E: ROC curve between AFP/ATV and post-op recurrence; 0.00, ATV < 13.78 cm^3 group; 1.00, ATV ≥ 13.78 cm^3 group; F: Correlation between AFP/ATV and DFS rates.

low differentiation subtype. With elevated AFP levels, such ratios were continuously increased. Therefore, elevation of pre-op AFP may further elevate the percentage of low to moderate tumor cells, affecting patient prognosis. Third, a previous study demonstrated significantly higher AFP expression in liver tumor tissues than peri-hepatic cancer [7]. Peripheral cell masses have stem cell-like properties, with substantial clonal proliferation, tumor formation, and self-renewal ability. Due to the naïve phenotype with AFP high expression, it can escape from body immune surveillance, causing tumor occurrence, progression, and metastasis. Therefore, AFP elevation indicates activity of peri-hepatic tumor cell masses, leading to a high incidence of post-op tumor recurrence and metastasis.

Table 4. Correlation between AFP levels and pathology differentiation types

Subgroup	Pathology differentiation			*P*	*r*
	Low	Moderate	High		
AFP					
20-200 ng/ml	14	48	5		
200-400 ng/ml	17	12	0	0.002	0.257
≥ 400 ng/ml	26	22	3		
ATV					
< 13.78 cm^3	60	113	68	0.161	0.081
≥ 13.78 cm^3	21	26	14		

Table 5. Relationship between ATV and pre-op AFP

Group	Pre-op AFP		*P*	*r*
	AFP positive	AFP negative		
< 13.78 cm^3	116	125	0.036	0.121
≥ 13.78 cm^3	31	30		

Due to the rough correlation between AFP and tumor sizes, the single application of AFP was not convincing. As a recently discovered prognostic evaluation index for HCC patients, ATV has relatively higher reliability in assessing tumor loading [4]. In this study, patients with ATV ≥ 13.78^3 had lower post-op DFS rates than those having lower than 13.78 cm^3 ATV. In summary, larger ATV indicates a heavier tumor load, worsened post-op body status, and higher recurrent rates. The current study also revealed a positive correlation between pre-op AFP and ATV, suggesting that larger tumor volume was correlated with higher AFP [8, 12, 13]. As mentioned previously, patients with higher AFPs showed worse prognosis. ROC curves predicted only 35.4% sensitivity of ATV, noting higher misdiagnosis rates for tumor recurrence. In practice, tumor morphology may be biased, significantly, due to infiltrative growth and irregular shapes. Thus, ATV still has limitations in predicting prognosis of HCC.

Previous studies have revealed the weakness of using AFP or ATV to predict prognosis of HCC patients. Toso et al. [14] showed relative worse prognosis of HCC patients having AFP ≥ 400 ng/mL and TTV ≥ 115 cm^3. The current study analyzed the correlation between AFP/ATV and prognosis of HCC patients in the BCLC-A stage. ROC curve analysis revealed AFP/ATV=21.72 as the best predictive boundary line for judging post-op recurrent risks for HCC patients in the BCLC-A stage, with 60.8% sensitivity and 79.1% specificity. HCC patients with less than 21.72 of AFP/ATV had higher DFS rates than those with higher than 21.72 of AFP/ATV. Therefore, AFP/ATV can work as a reference index evaluating the prognosis of HCC patients in the BCLC-A stage, as reported by Lee et al. [15]. ROC curves revealed relatively higher sensitivity and specificity levels using the AFP/ATV predictive line. Therefore, using AFP/ATV to predict prognosis of HCC patients in the BCLC-A stage has certain clinical value.

In summary, pre-op AFP, ATV, pre-op ALT, and integrity of tumor capsule are independent risk factors for prognosis. HCC patients in the BCLC-A stage with AFP ≥ 400 ng/mL, ATV ≥ 13.78 cm^3, and AFP/ATV ≥ 21.72 had a relatively worse prognosis. Pre-op AFP/ATV ratios have been shown to be crucial indexes in predicting post-op recurrent risks for HCC patients in the BCLC-A stage.

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Disclosure of conflict of interest

None.

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