



Serum α -fetoprotein level as a biomarker for early recurrence after R0 resection in primary hepatocellular carcinoma

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Abstract

Objectives: To investigate the impact of serum α -fetoprotein (AFP) levels on survival and early recurrence after R0 resection in primary hepatocellular carcinoma (HCC).

Methods: We retrospectively analyzed clinical data of 582 patients with primary HCC (male: 488, female: 94; mean age: 51 years [age range, 31–73 years]) who underwent R0 resection between January 1997 and December 2009. The 1-, 3-, and 5-year overall survival (OS) rates and the clinicopathological factors between the AFP-negative and AFP-positive groups were compared. Risk factors of early recurrence in the AFP-positive group were further analyzed.

Results: Age and histological differentiation were significantly lower in the AFP-positive group than in AFP-negative group ($\chi^2=11.004, 32.293; P=0.000$). In the AFP-positive group, serum γ -glutamyl transferase levels, tumor diameters, TNM stage, and early recurrence rate were higher ($\chi^2=9.814$ to $14.009, P<0.05$), whereas the proportion of solitary HCC was lower ($\chi^2=8.509, P=0.004$). The 1-, 3-, and 5-year OS rates in the AFP-positive group were 80.5%, 66.9%, and 40.2%, respectively, and those in the AFP-negative group were 89.2%, 79.9%, and 50.3%, respectively ($\chi^2=11.884, P=0.001$). The 1-, 3-, and 5-year disease-free survival rates were 65.6%, 48.5%, 29.6% in the AFP-positive group and 81.7%, 63.9%, 42.1% in the AFP-negative group respectively ($\chi^2=15.574, P=0.000$). The median OS times of early recurrence and non-early recurrence were 10 and 62 months, respectively ($\chi^2=45.013, P=0.000$), and their median survival times from recurrence to death were 6 and 14 months, respectively ($\chi^2=40.581, P=0.000$). Multiple-factor analysis suggested non-solitary HCC and low histological differentiation are independent risk factors influencing early recurrence of HCC.

Conclusion: Elevated preoperative serum AFP levels were indicative of early HCC recurrence.

Keywords: Carcinoma, Hepatocellular, Alpha-Fetoproteins, Hepatectomy, Local neoplasm recurrence

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Introduction

Liver transplantation, liver resection, and radiofrequency ablation are effective means for the treatment of hepatocellular carcinoma (HCC), but the postoperative recurrence rate is high, which is an important factor affecting the

prognosis of surgical treatment [1,2]. The level of serum alpha-fetoprotein (AFP) increases in 60% - 80% of HCC patients, indicating that AFP may be an important prognostic factor for HCC, but this is yet to be confirmed [3]. We retrospectively reviewed the data of 582



patients with HCC who underwent R0 resection to explore the relationship between AFP level and the prognosis after R0 resection.

Methods

Patients

Patients with primary HCC who received operation therapy in our hospital from January 1997 to December 2009 were selected. According to the concept of solitary large hepatocellular carcinoma proposed by Yang et al. [4], solitary HCC was defined as a single tumor with complete tumor capsule or false capsule and no visible epidural invasions (i.e. no sub foci or satellite lesions, no tumor rupture, no visible vascular invasion or regional lymph node metastasis). Exclusion criteria included a loss to follow-up ($n=47$ [6.9%]), death within 30 postoperative days ($n=10$ [1.47%]), no R0 resection ($n=23$ [3.38%]), and no preoperative AFP data ($n=19$ [2.79%]).

A total of 582 patients were analyzed retrospectively, including 488 males and 94 females, aged between 31–73 years old, with a mean age of 51 years. There were 323 cases (positive group) with preoperative AFP $>20\mu\text{g/L}$, and 259 cases (negative group) with AFP $\leq 20\mu\text{g/L}$. This study was approved by the institute's ethics committee.

Surgical procedure

Hepatectomy was performed under the following conditions: with proper Child-Pugh and hepatic function reserve status (Model for End-Stage Liver Disease [MELD] score); and no more than 3 tumors, no distant metastasis, and no tumor invasion to the main portal vein.

No residual tumor in residual liver confirmed by ultrasound during the operation and negative margin confirmed by pathology were regarded as radical hepatic resection. Under proper condition of hepatic functional reserve of patients, liver sections were localized with ultrasound during surgery using the Couinaud segmentation method, and anatomical liver resection was performed; other resection methods such as local resection and tumor enucleation were regarded as nonanatomical liver resection.

Follow-up

Within 3 postoperative months, a liver ultrasound scan, liver function test, and serum AFP levels test were performed monthly. Serum AFP was detected by rocket immunoelectrophoresis (before 2005) and electrochemiluminescence (after 2006). For patients with an operation margin <1 cm, satellite nodules, and vascular invasion, transcatheter arterial chemoembolization was performed at 3-4 postoperative weeks. Subsequent reexamination was performed every 3 months. The follow-up of patients with recurrence ended on the diagnosis of recurrence proved by abdominal computed tomography (CT), lung CT and/or hepatic arterial lipiodol angiography, while the follow-up of patients with no recurrence ended on December 31, 2011, or at death.

Statistical analysis

SPSS 13 statistical software was applied for data analyses. Comparison of the study variables between groups was performed using χ^2 test or F test. The Kaplan-Meier method and Log-

rank test were used for survival analysis. The single factor with P-value lower than 0.05 was introduced into the Cox proportional hazard regression model. $P<0.05$ was considered to be statistically significant.

Results

The relationship between the serum AFP concentration and clinical pathological factors

Univariate analysis showed that the proportions of older age, high histological differentiation, and solitary HCC in the positive group were significantly lower than those in the negative group, while the proportions of large HCC and TNM stage II-IV were significantly higher in the positive group than those in the negative group. There was no significant difference on the prevalence of hepatitis or cirrhosis between the two groups (Table 1).

The relationship between serum AFP concentration and survival

The follow-up time ranged from 2.0–167.7 months, with a median of 36.4 months. The 1-, 3-, and 5-year overall survival (OS) rates of the positive group and the negative group were 80.5%, 53.9%, 40.2% and 89.2%, 69.8%, 50.3%, respectively ($\chi^2=11.884$, $P=0.001$). The 1-, 3-, and 5-year disease-free survival (DFS) rates were 65.6%, 38.4%, 29.6% and 81.7%, 51%, 42.1% respectively ($\chi^2=15.574$, $P=0.000$). The postoperative OS rate and DFS rates of the positive group were lower than those of the negative group (Figure 1). The median OS times were 10 and 62 months for patients with early recurrence



and patients with non-early recurrence, respectively ($\chi^2 = 45.013$, $P=0.000$). The median survival times from recurrence to death were 6 and 14 months for patients with early recurrence and patients with non-early recurrence, respectively ($\chi^2 = 40.581$, $P=0.000$).

The relationship between the serum AFP concentration and early recurrence

At the end of the follow-up, there were 355 patients with HCC recurrence or metastasis, including 109 cases of early recurrence within 6 months (30.7%). The rate of early recurrence was significantly different between the positive group (78 cases, 36.1%) and the negative group (31 cases, 22.3%) ($\chi^2 = 7.580$, $P=0.007$).

Univariate analysis showed that, in the AFP positive group, there were significant differences between patients with early recurrence and patients with non-early recurrence on γ -glutamyl transpeptidase levels ($\chi^2 = 5.323$, $P=0.025$), tumor diameter ($\chi^2 = 24.506$, $P=0.000$), liver capsule invasion ($\chi^2 = 6.106$, $P=0.013$), histological differentiation ($\chi^2 = 7.152$, $P=0.028$), TNM stage ($\chi^2 = 36.003$, $P=0.000$), and solitary HCC ($\chi^2 = 46.902$, $P=0.000$), but no significant difference on gender, age, platelet count, albumin, ALT, cirrhosis, viral hepatitis, Child-Pugh grade, or portal hypertension ($\chi^2 = 0.014 \sim 4.569$, $P=0.206 \sim 0.882$). A Cox proportional hazard model was introduced to conduct multivariate analysis on the significant variables above, and the results showed that non-solitary HCC and low histological differentiation were independent risk factors for

Table 1. Preoperative alpha-fetoprotein (AFP) levels and clinical pathological factors in patients with hepatocellular carcinoma (n, %)

Factors	AFP > 20 $\mu\text{g/L}$	AFP \leq 20 $\mu\text{g/L}$	χ^2	P
Cases	323	259	-	-
Gender			1.199	0.308
Male	266 (82.4)	222 (85.7)		
Female	57 (17.6)	37 (14.3)		
Age			11.004	0.000
≤ 60	253 (78.3)	171 (66.0)		
> 60	70 (21.7)	88 (34.0)		
γ -GGT ¹ (U/L)			11.212	0.001
< 65	172 (55.5)	172 (69.4)		
≥ 65	138 (44.5)	76 (30.6)		
Platelet ^a ($\times 10^9/\text{L}$)			2.877	0.106
< 100	81 (25.5)	48 (19.4)		
≥ 100	237 (74.5)	199 (80.6)		
Albumin (g/L) ²			0.787	0.399
< 35	48 (14.9)	32 (12.4)		
≥ 35	274 (85.1)	227 (87.6)		
ALT (U/L)			1.148	0.298
< 60	233 (72.1)	197 (76.1)		
≥ 60	90 (27.9)	62 (23.9)		
Cirrhosis			4.007	0.051
No	25 (7.7)	33 (12.7)		
Yes	298 (92.3)	226 (87.3)		
Viral hepatitis			6.228	0.101
Negative	27 (8.4)	37 (14.3)		
Hepatitis B	287 (88.9)	218 (84.2)		
Hepatitis C	6 (1.9)	2 (0.8)		
Hepatitis B+C	3 (0.9)	2 (0.8)		
Child-Pugh grading			0.087	0.854
A	305 (94.4)	246 (95)		
B	18 (5.6)	13 (5.0)		
Portal hypertension ³			2.570	0.111
No	264 (82)	225 (86.9)		
Yes	58 (18.0)	34 (13.1)		
Tumor diameter (cm)			12.100	0.001
≤ 5	149 (46.1)	157 (60.6)		
> 5	174 (53.9)	102 (39.4)		
Liver capsule invasion			2.483	0.123



Factors	AFP> 20 µg/L	AFP ≤ 20 µg/L	χ ²	P
No	89 (27.6)	87 (33.6)		
Yes	234 (72.4)	172 (66.4)		
Histological differentiation			32.293	0.000
High	21 (6.5)	50 (19.3)		
Middle-low	295 (91.3)	191 (73.7)		
Necrosis	7 (2.2)	18 (6.9)		
TNM staging			9.814	0.002
I	204 (63.2)	195 (75.3)		
II–IV	119 (36.8)	64 (24.7)		
Solitary			8.509	0.004
No	122 (37.8)	69 (26.6)		
Yes	201 (62.2)	190 (73.4)		
Recurrence during follow-up			10.536	0.001
No	107 (33.1)	120 (46.3)		
Yes	216 (66.9)	139 (53.7)		
Early recurrence			14.009	0.000
No	138 (63.9)	108 (77.7)		
Yes	78 (36.1)	31 (22.3)		

Note: ALT: alanine transaminase; early recurrence referred to recurrence within 6 postoperative months; - : No data were available; a the platelets of the patients who underwent splenic resection were not included;¹ the data of 24 cases were excluded;² the data of 1 case was excluded;³ the data of 1 case was excluded

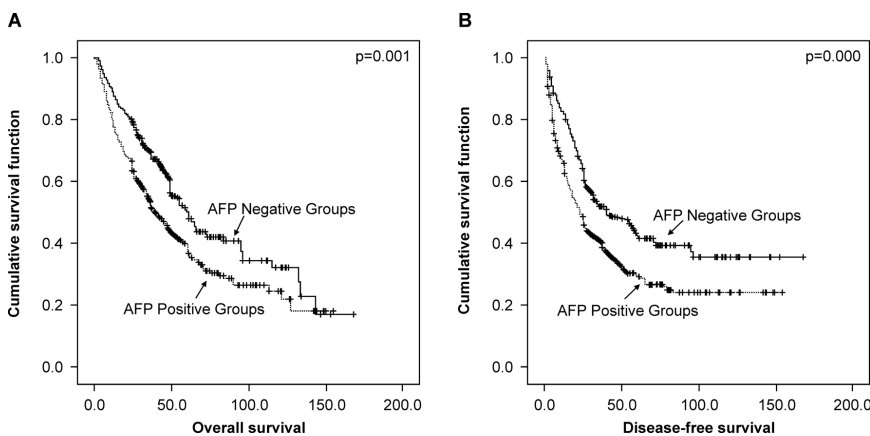


Fig. 1. Survival curves of patients with HCC in the preoperative AFP positive group and negative group A) Overall survival rate B) Disease-free survival rate

early recurrence in HCC patients (hazard ratio [HR] =3.712, $P=0.005$; $HR=1.477$, $P=0.000$).

In patients with early recurrence, the median OS time of the positive group was significantly longer than that of

the negative group (11 vs. 9 months, $\chi^2 =5.943$, $P=0.015$), and the median time from recurrence to death of positive group was also significantly longer than that of negative group (6 vs. 5 months) ($\chi^2 =6.237$, $P=0.013$). However, in patients with non-early recurrence, the median OS time of positive group was significantly shorter than that of negative group (61 vs. 72 months, $\chi^2 =4.192$, $P=0.041$), while no significant difference was noted on the median times from recurrence to death between the two groups ($\chi^2 =2.227$, $P=0.136$).

According to preoperative and postoperative AFP levels, 141 patients with recurrent HCC were divided into 4 groups: group A (preoperative and postoperative AFP ≤ 20 µg/L), group B (preoperative AFP ≤ 20 µg/L, postoperative AFP > 20 µg/L), group C (preoperative AFP > 20 µg/L, postoperative AFP ≤ 20 µg/L), and group D (preoperative and postoperative AFP > 20 µg/L). To investigate the relationship of preoperative and postoperative AFP levels with solitary HCC and survival among patients with recurrence, the distribution of solitary HCC and the survival times were compared among the four groups (Table 2). The OS time and DFS time of group D were significantly shorter than all other three groups ($P < 0.01$), and there were no significant differences on these two variables among group A, B, and C ($P > 0.05$). All 7 cases of early recurrence in group A and 23 cases (23/31) of early recurrence in group D were with non-solitary HCC ($\chi^2 =8.025$, $P=0.045$).

Discussion

A high postoperative recurrence rate is



Table 2. The relationship of preoperative and postoperative alpha-fetoprotein (AFP) levels with solitary hepatocellular carcinoma (HCC) and survival among patients with recurrence

Group	Cases	Solitary HCC (cases)	Disease-free survival (months)	Overall survival (months)
A	34	19	16.0 ± 4.1	49 ± 5
B	12	11	22.0 ± 2.6	57 ± 9
C	19	15	22.0 ± 2.2	60 ± 14
D	76	35	9.0 ± 1.4	26 ± 4
$F(\chi^2)$	-	13.328	17.579	12.389
P	-	0.004	0.001	0.006

Note: No data are available; χ^2 : the statistical value of group comparison of enumeration data, F : the statistical value of group comparison of measurement data.

a major factor leading to poor long-term survival of patients with resected HCC [5]. However, reports in the literature for early HCC recurrence within 6 postoperative months are limited. In this study, the early recurrence rate was 18.7%, accounting for 30.7% of the patients with recurrence. The median OS time and the time from recurrence to death of patients with non-early recurrence were 62.7 and 13.6 months, respectively; while those of patients with early recurrence were only 10.4 and 6.5 months, respectively. This suggests that the patients with early recurrence demonstrate a faster disease progression than the patients with non-early recurrence. It is likely that the prediction and prevention of early recurrence in patients with HCC has vital significance for the postoperative course.

AFP level is an effective tumor marker for monitoring the recurrence of HCC [6]. Moriguchi et al. [7] found that serum AFP > 20 µg/L was one of the risk factors of death caused by early recurrence after HCC radical resection. In addition, a high serum AFP level in patients with HCC indicates low tumor differentiation, larger tumor diameter, high

recurrent likelihood, and a poor prognosis after resection [8-9]. Here, the results of univariate analysis between the positive and negative group are consistent with the results of these previous studies. According to the survival analyses and multivariate analyses, the elevated preoperative serum AFP level increases the likelihood of early recurrence particularly in patients with high-histological-differentiated and non-solitary HCC. In these patients, postoperative systematic treatment should be actively conducted, and strict monitoring of AFP levels and regular physical examination should be done to facilitate timely discovery and early intervention.

Univariate analysis showed that there were significantly more patients with increased AFP levels in the non-solitary HCC group compared with the solitary HCC group, and that the prognosis of non-solitary HCC was very poor. This suggests that patients should be screened for non-solitary HCC through a combination of an imaging examination and AFP levels, and those patients with non-solitary HCC should be treated carefully. As shown

in Table 2, most recurrences in group A and D were caused by monocentric origin, and happened earlier; while most recurrences in the B and C groups were of multicentric origin, the first primary of which was mainly solitary HCC. Accordingly, it suggests that the preoperative and relapsing serum AFP concentrations, and that whether HCC is solitary or not could be used to predict the origin and time of recurrence and guide the treatment. Analyzing of the relationships between various clinical pathological features and the recurrence of the disease provides a better prognostic indicator than just analyzing such factors separately. It was noted that, in early recurrent cases, the OS time of the positive group was higher than that of negative group. Further analysis showed that the median time from recurrence to death of the former was longer than that of the latter. It may indicate that for patients with elevated preoperative AFP levels, AFP may gradually decrease to the normal concentrations after R0 resection, and the postoperative increases in AFP precede the imaging-based detection of tumor recurrence. Therefore, monitoring postoperative AFP levels in these patients could lead to a more timely diagnosis and treatment of recurrence. In addition, AFP-positive patients has a higher early recurrence rate, but as it is easier to obtain timely postoperative monitoring and treatment, the survival rate is higher than that of patients with negative AFP.

In summary, elevated preoperative serum AFP levels could predict the early recurrence of HCC. For HCC patients, the preoperative and postoperative



serum AFP levels and solitary tumor, especially combined with the results of regular physical and imaging examinations, might be very useful in forecasting the recurrent origin and timing, and in guiding appropriate treatment. Utilizing this method, the early discovery and treatment of recurrent HCC can be fully achieved, which has important significance in improving the prognosis of HCC patients.

Competing interests

The authors declare no competing interests.

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. COCHRANE UPDATES & NICE GUIDELINES .

PHYSICAL ACTIVITY: BRIEF ADVICE FOR ADULTS IN PRIMARY CARE

This guidance aims to support routine provision of brief advice on physical activity in primary care practice. The recommendations cover:

- identifying adults who are inactive
- delivering and following up on brief advice
- incorporating brief advice in commissioning
- systems to support brief advice
- information and training to support brief advice.

The guidance is for commissioners of health services and anyone working in primary care whose remit includes offering lifestyle advice. Examples include: exercise professionals, GPs, health trainers, health visitors, mental health professionals, midwives, pharmacists, practice nurses, physiotherapists.

It may also be of interest to others with a role in encouraging physical activity and members of the public.

(Source: NICE Public health guidance, PH44, May 2013; available at <http://guidance.nice.org.uk/PH44>)