RESEARCH COMMUNICATION

Distinctions Between Clinicopathological Factors and Prognosis of Alpha-fetoprotein Negative and Positive Hepatocelluar Carcinoma Patients

Jia Xu, Chang Liu*, Lei Zhou, Feng Tian, Ming-Hui Tai, Ji-Chao Wei, Kai Qu, Fan-Di Meng, Ling-Qiang Zhang, Zhi-Xin Wang, Jing-Yao Zhang, Hu-Lin Chang, Si-Nan Liu, Xin-Shen Xu, Yan-Zhou Song, Jun Liu, Peng Zhang

Abstract

Serum alpha-fetoprotein (AFP) is a significant marker for clinical diagnosis and prognosis evaluation in hepatocellular carcinoma (HCC) patients. However, some proportion of liver cancer patients are AFP-negative (AFP ≤20ng/ml). In order to study the differences between clinicopathological factors and prognosis of alpha-fetoprotein negative and positive patients, a total of 114 cases (41 AFP-negative and 73 AFP-positive) were selected for our research. By systematically statistical analysis, the results demonstrated that compared with AFP-negative patients, AFP-positive examples were more likely to feature cirrhosis nodules, non-complete neoplasm capsules, and a poor Edmondson-steiner grade. Furthermore, AFP-negative patients demonstrated a favorable long-term prognosis. By univariate analysis and multivariate analysis with Cox's proportional hazards model, multiple tumors were found to be independent risk factors for worse survival of AFP negative patients; however, less tumor-free margins, multiple tumors and Edmondson-steiner grades III/IV, proved to be independent risk factors leading to a poor prognosis of AFP positive cases. Finally, we can infer that high levels of AFP signify a highly malignant tumor and unfavorable prognosis.

Keywords: Clinicopathological factors - prognosis - alpha-fetoprotein status - hepatocellular carcinoma

Asian Pacific J Cancer Prev, **13**, 559-562

Introduction

From a global cancer statistics in 2011, hepatocellular carcinoma (HCC) in men is the fifth most frequently diagnosed cancer and the second most frequent cause of cancer mortality all over the world. In women, it is the seventh most commonly diagnosed cancer and the sixth important cause of cancer death. More than half of these cases and deaths were estimated to occur in China (Jemal et al., 2011).

In 1963, alpha-fetoprotein (AFP) was first found in the serum of mice suffering from liver cancer; Soon afterwards it was detected in the serum of hepatocellular carcinoma patients in 1964 (Alpert et al., 1968; Abelev et al., 1971). From then on, AFP, as a generally accepted tumor marker, especially used for diagnosis of HCC, was gradually concerned by us. In previous studies, large amounts of alpha-fetoprotein were found in fetal liver and developing yolk sac; In addition to, there were smaller amounts of alpha-fetoprotein observed in almost the entire gastrointestinal tract of human conceptus (Gitlin et al., 1972). So human alpha-fetoprotein is the normal product only during gestation. It is synthesized by developing liver beginning at 6 weeks of gestation but the synthesis will cease at or near birth, and then the concentration will decline to a low level (less than 10ng/ml). In adults, under normal conditions, serum AFP concentration is below 20 ng/ml (Gitlin et al., 1966; Debruyne et al., 2008).

AFP synthesis is repressed after birth. If there is an abnormal increase, it implies the occurrence of some certain disease, such as hepatocellular carcinoma and embryonal cell carcinoma. Nowadays, AFP is generally used not only in screening and diagnosing HCC, but also in evaluating the prognosis of HCC patients who undergo the hepatectomy (Peng et al., 2004). Although AFP has occasionally been reported in some other primary tumor e.g. gastric carcinoma, it was generally thought to be specific for hepatocellular carcinoma and embryonal cell carcinoma (Abelev et al., 1971; Chang et al., 1992).

With the development of imaging techniques, the prevalence of HCC patients not associated with a significant elevation of serum AFP (<20ng/ml) is gradually increasing. We defined them as AFP-negative patients. According to statistics, about 40% of patients with early

Department of Hepatobiliary Surgery, the First Affiliated Hospital of Medical College, Xi'an Jiaotong University, Xi'an, China
**For correspondence: liuchangdoctor@163.com*

Table 1. Clinicopathological Factors of Alpha-fetoprotein Negative and Positive HCC Patients

clinicopathologic factors	AFP-negative (n=41) <=20ng/ml	AFP-positive (n=73) >20ng/ml	P value
Age(years)	52±11	55±12	0.18
Gender	Male n=29	Male n=60	0.16
	Female n=12	Female n=13	
Cirrhosis nodules	With n=27	With n=60	0.04
	Wihtout n=14	Wihtout n=13	
GGT(u/L)	84±110	111±158	0.33
ALB (g/L)	40±5.0	39±4.7	0.42
TBIL (mmmol/L)	16±7	25±48	0.2
ALP(u/L)	102±60	126±90	0.12
PT (s)	12.9±1.5	13.1±1.8	0.56
HBsAg	positive n=28	positive n=55	0.42
	negative n=13	negative n=18	
Tumor distribution	Within a segment or a lobe n=31	Within a segment or a lobe n=52	0.61
	Invade the semi-liver or the whole liver n=10	Invade the semi-liver or the whole liver n=21	
Tumor size	Small HCC n=23	Small HCC n=35	0.4
	Non-small HCC n=18	Non-small HCC n=38	
Tumor number	Single tumor n=29	Single tumor n=46	0.41
	Multiple tumor n=12	Multiple tumor n=27	
Capsule	Complete capsule n=19	Complete capsule n=12	<0.01
	Non-complete capsule n=22	Non-complete capsule n=61	
Resection mode	Radical resection n=29	Radical resection n=48	0.59
	Palliative resection n=12	Palliative resection n=25	
TNM stage	stage I or II n=29	stage I or II n=46	0.41
	stage III n=12	stage III n=27	
Edmondson-steiner grade	Grade 1 or 2 n=25	Grade 1 or 2 n=28	0.02
	Grade 3 or 4 n=16	Grade 3 or 4 n=45	

HCC and 15-20% of patients with advanced HCC show the normal AFP levels. In China, about 30-40% of HCC patients are AFP negative (Maringhini et al., 1988; Lok et al., 1989). So, it is necessary to know well about the clinical features of AFP-negative patients, compared with those of AFP-positive cases.

In this study, we collected and analyzed the clinical and follow-up information of AFP-negative patients. Some of similar AFP-positive patients' information, as a control group, was also included in. Based on it, we expect to reveal the differences of clinicopathological factors and prognosis in two groups.

Materials and Methods

Patients

A total of 114 HCC patients, of which 41 AFP-negative (AFP≤20ng/ml) and 73 AFP-positive (AFP>20ng/ml), from Aug. 1st 2002 to Dec. 30th 2009 in the First Affiliated Hospital of Medical College, Xi'an Jiao tong University, were include in this study. All of them were diagnosed with primary liver cancer by preoperative computed tomography (CT) scan, ultrasonography, AFP and other tests. Based on the assessment of general condition and preoperative hepatic functional reserve, all patients in this study underwent the hepatectomy. The postoperative pathology confirmed the previous diagnosis.

There are two ways of hepatectomy, namely, radical resection and palliative resection. All the patients did not undergo any other type of palliative treatment e.g. sorafenib or TACE. After discharge, all patients were followed up regularly to observe their survival status over time.

Clinicopathologic factors

We analyzed a series of clinicopathologic factors may relate with the levels of AFP. All factors were categorized as Patients' general data such as age and gender; preoperative biochemical data such as glutamyl transpeptidase (GGT) value, albumin (ALB) value, total bilirubin (TBIL) value, alkaline phosphatase (ALP) value, prothrombin time (PT), hepatitis B surface antigen (HBsAg) status and cirrhosis nodules status(whether the patients had the cirrhosis nodules or not); Intraoperative information such as tumor distribution (segment , lobe, semi-liver and whole liver four-level), tumor size (small HCC: 5 cm or less single nodule or up to three lesions of 3 cm or less, or Non small HCC) (Ryder et al., 2003), tumor number (single or multiple tumor), capsule status (complete or non-complete capsule) and resection mode (radical or palliative resection); Postoperative pathology data such as TNM clinical staging and Edmondson-steiner grading.

Statistical analysis

All the statistical data were processed by spss16.0. The analysis of measurement data were carried out using Student's T-test, as well as enumeration data using Chi-square test. The survival analysis was calculated by Kaplan Meier method. Comparison of survival in different groups was performed by the log-rank test. Each of covariates was analyzed by univariate Cox's proportional hazards model, and removed the no statistically significant covariates, and then the valid variables were analyzed by multivariate Cox's proportional hazards model. $P < 0.05$ was considered significantly different for all statistical results.

Table 2. Respective Risk Factors Affecting the Survival of AFP Negative and Positive Patients

Clinicopathologic factors	AFP-negative (n=41)<=20ng/ml	Univariate analysis	Multivariate analysis	AFP-positive (n=73)>20ng/ml	Univariate analysis	Multivariate analysis
Age(years)	52±11	0.28		55±12	0.06	
Gender	Male n=29	0.59		Male n=60	0.19	
	Female n=12			Female n=13		
Cirrhosis nodules	With n=27	0.05		With n=60	0.55	
	Wihtout n=14			Wihtout n=13		
GGT(u/L)	84±110	0.83		111±158	0.3	
ALB (g/L)	40±5.0	0.27		39±4.7	0.14	
TBIL (mmmol/L)	16±7	0.91		25±48	0.57	
ALP(u/L)	102±60	0.44		126±90	0.14	
PT (s)	12.9±1.5	0.09		13.1±1.8	0.52	
HBsAg	positive n=28	0.24		positive n=55	0.16	
	negative n=13			negative n=18		
Tumor-free margin (cm)	0.56±0.67	0.05		0.56±0.66	<0.01	0.02
Tumor distribution	Within a segment or a lobe n=31	0.03	0.39	Within a segment or a lobe n=52	0.01	0.43
	Invade the semi-liver or the whole liver n=10			Invade the semi-liver or the whole liver n=21		
Tumor size	Small HCC n=23	0.07		Small HCC n=35	0.01	0.31
	Non-small HCC n=18			Non-small HCC n=38		
Tumor number	Single tumor n=29	0.01	0.02	Single tumor n=46	<0.01	<0.01
	Multiple tumor n=12			Multiple tumor n=27		
Capsule	Complete capsule n=19	0.04	0.2	Complete capsule n=12	0.04	0.27
	Non-complete capsule n=22			Non-complete capsule n=61		
Resection mode	Radical resection n=29	0.01	0.07	Radical resection n=48	0.01	0.19
	Palliative resection n=12			Palliative resection n=25		
TNM stage	stage I or II n=29	0.04	0.39	stage I or II n=46	0.04	0.62
	stage III n=12			stage III n=27		
Edmondson-steiner grade	Grade 1 or 2 n=25	0.28		Grade 1 or 2 n=28	<0.01	<0.01
	Grade 3 or 4 n=16			Grade 3 or 4 n=45		

Results

Comparison of clinicopathological factors in two groups of patients

The distinctions between clinicopathological factors of AFP negative and positive hepatocelluar carcinoma patients were analyzed, and the results were shown in Table 1. According to it, we can conclude that whether having cirrhosis nodules and existing a complete capsule or not was associated with the levels of AFP. Furthermore, the serum AFP concentration was also correlated with Edmondson-steiner Grading. (All p <0.05) whereas, there were some conclusions obtained differently from other authors' view, such as tumor size, TNM stages and gender (Farinati et al., 2006), no significant distinctions were found in different AFP levels (All p>0.05).

Comparison of survival in two groups of patients

The survival analysis of two groups of patients was calculated by Kaplan Meier method, and the result was shown in Figure 1. The survival rate between the two groups was considered statistically different by log-rank test (p=0.029).

Respective risk factors affecting the survival of AFP negative and positive patients

A series of possible risk factors were identified by Cox's proportional hazards model (Table 2). By univariate analysis, the risk factors for poor survival of AFP negative patients were the tumor distribution, tumor number, capsule status, resection mode and TNM stage;

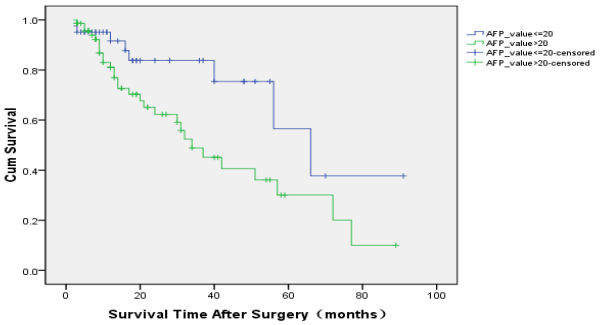

Figure 1. Cumulative Survival Curves of AFP Negative and Positive Patients

the tumor-free margin, tumor distribution, tumor size, tumor number, capsule status, resection mode, TNM stage and Edmondson-steiner grade were considered as the risk factors for worse survival of AFP positive ones (All p<0.05). After that, we conducted multivariate analysis to them. We found that multiple tumor, as the significant independent risk factors, lead to the unfavorable prognosis of AFP negative patients. Corresponding to former, the significant independent risk factors for poor survival of AFP positive ones were the less tumor-free margin, multiple tumor and Edmondson-steiner grade III/IV (All p<0.05).

Discussion

Through the above series of results we have got, we can found that there existed a significant association between AFP levels and some clinicopathologic factors, they were

cirrhosis nodules, capsule status and Edmondson-steiner grading, as listed in the Table 1. The patients with high AFP levels are more likely to accompany with the cirrhosis nodules, the non-complete neoplasm capsule and the poor Edmondson-steiner grade. As a result of known, cirrhosis is a risk factors affecting the occurrence and progression of HCC (Velázquez et al., 2003), and most malignant tumors have a non-complete capsule, and a worse evaluation by Edmondson-steiner grading (Zhou et al., 2008). Therefore, we can infer that high levels of AFP were perhaps associated with the highly malignant tumor and poor prognosis. This inference was just confirmed by the result of survival analysis we have got in Figure 1.The mechanism why exists such a relation is still uncertain, some possible explanations were proposed as follows, high AFP levels correlate with p53 mutation (Peng et al., 2004); High AFP levels can stimulate tumor cell sustained growth (Wang et al., 1999); The hepatoma cells can escape from immune surveillance in high AFP levels (Nagao et al., 2000; Li et al., 2005). Moreover, by multivariate analysis, we can see that multiple tumor can bring about the worse prognosis for AFP-negative patients, and AFP-positive patients with less tumor-free margin, multiple tumor and Edmondson-steiner grade III/IV also tend to have the unfavorable survival. Multiple tumor means multiple violations of tumor cells; Less tumor-free margin means that residual tumor cells are more likely to exist in the remnant liver after hepatectomy; Edmondson-steiner grade III/IV means poorly differentiated and highly malignant tumors. All of them can increase the likelihood of tumor metastasis and recurrence.

In summary, the clinicopathological factors and prognosis of alpha-fetoprotein negative and positive hepatocelluar carcinoma patients are different from each other. Although sometimes Alpha-fetoprotein test displays the negative and false-positive results (Lok et al., 1989), the false diagnosis can be avoided through combination with other detection methods such as, imaging techniques or some other tumor markers testing (Fujiyama et al., 2002). Therefore, AFP is still a valuable marker for clinical assessment of HCC patients.

References

Alpert ME, Uriel J, de Nechaud B (1968). Alpha1 fetoglobulin in the diagnosis of human hepatoma. *N Engl J Med*, **278**, 984-6.

Abelev G (1971). Alpha-fetoprotein in ontogenesis and its association with malignant tumors. *Adv Cancer Res*, **14**, 295-358.

Chang YC, Nagasue N, Abe S, et al (1992). Comparison between the clinicopathologic features of AFP-positive and AFP-negative gastric cancers. *Am J Gastroenterol*, **87**, 321.

Debruyne EN, Delanghe JR (2008). Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. *Clinica Chimica Acta*, **395**, 19-26.

Fujiyama S, Tanaka M, Maeda S, et al (2002). Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. *Oncology*, **62**, 57.

Farinati F, Marino D, De Giorgio M, et al (2006). Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? *Am J Gastroenterol*, **101**, 524-32.

Gitlin D, Boesman M (1966). Serum alpha-fetoprotein, albumin, and gamma-G-globulin in the human conceptus. *J Clinic Invest*, **45**, 1826.

Gitlin D, Perricelli A, Gitlin GM (1972). Synthesis of -fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. *Cancer Res*, **32**, 979-82.

Jemal A (2011). Global Cancer Statistics (vol 61, pg 69, 2011). *Ca-Cancer J Clin*, **61**, 134.

Lok ASF, Lai CL (1989). α-fetoprotein monitoring in chinese patients with chronic hepatitis B virus infection: Role in the early detection of hepatocellular carcinoma. *Hepatology*, **9**, 110-5.

Li MS, Ma QL, Chen Q, et al (2005). Alpha-fetoprotein triggers hepatoma cells escaping from immune surveillance through altering the expression of Fas/FasL and tumor necrosis factor related apoptosis-inducing ligand and its receptor of lymphocytes and liver cancer cells. *World J Gastroenterol*, **11**, 2564-9.

Maringhini A, Cottone M, Sciarrino E, et al (1988). Ultrasonography and alpha-fetoprotein in diagnosis of hepatocellular carcinoma in cirrhosis. *Dig Dis Sci*, **33**, 47-51.

Nagao M, Nakajima Y, Kanehiro H, et al (2000). The impact of interferon gamma receptor expression on the mechanism of escape from host immune surveillance in hepatocellular carcinoma. *Hepatology*, **32**, 491-500.

Peng SY, Chen WJ, Lai PL, et al (2004). High α-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: Significance of hepatitis virus infection, age, p53 and β-catenin mutations. *Int J Cancer*, **112**, 44-50.

Ryder S (2003). Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. *Gut*, **52**, iii1.

Velázquez RF, Rodríguez M, Navascués CA, et al (2003). Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. *Hepatology*, **37**, 520-7.

Wang XW, Xie H (1999). Significance of a-fetoprotein in the development of novel therapeutic agents. *Drugs of the Future*, **24**, 55-66.

Zhou L, Rui JA, Ye DX, et al (2008). Edmondson-Steiner grading increases the predictive efficiency of TNM staging for long-term survival of patients with hepatocellular carcinoma after curative resection. *World J Surg*, **32**, 1748-56.