RESEARCH ARTICLE

Prognostic Value of Serum AFP, AFP-L3, and GP73 in Monitoring Short-term Treatment Response and Recurrence of Hepatocellular Carcinoma after Radiofrequency Ablation

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Abstract

Purpose: Alpha-fetoprotein (AFP), Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), and Golgi protein 73 (GP73) levels have been widely used as tumor markers for the diagnosis of hepatocellular carcinoma (HCC). The aim of this study was to investigate whether these tumor markers could be used to monitor short-term treatment response and recurrence of HCC in patients undergoing radiofrequency ablation (RFA). Methods: Between July 2012 and July 2013, 53 consecutive patients with newly diagnosed HCC were prospectively enrolled in this study. Among these, 32 patients underwent RFA, after which they were followed up prospectively at the First Hospital of Jilin University in China. Results: AFP, AFP-L3, and GP-73 values pre-RFA were not associated with tumor size, whereas AFP and GP-73 levels tended to be associated with tumor number, the presence of vascular invasion, deterioration of liver function, advanced-stage disease, and a poor performance status. GP-73 levels were dramatically elevated in the patients with hepatitis C-associated HCC. Neither pre-RFA nor 1-month post-RFA tumor marker values were associated with short-term outcome. The short-term recurrence rate of AFP-positive patients measured 1 month post-RFA was obviously higher than that of AFP-negative patients. Conclusions: AFP and GP-73 values were associated with clinical variables representing tumor growth and invasiveness, and the AFP value measured 1 month post-RFA was a strong predictor of short-term recurrence in patients with HCC.

Keywords: AFP - AFP-L3 - GP-73 - hepatocellular carcinoma - radiofrequency ablation

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and third most common cause of cancer-related death worldwide (El–Serag and Rudolph, 2007), and its incidence is increasing in various countries (Bosetti et al., 2008; Chong et al., 2013). In China, 7.18% of the entire population carry the hepatitis B virus, and Chinese patients account for >55% of new cases of HCC worldwide. Notably, HCC is a leading cause of cancer-related death in China (Shariff et al., 2009; Garcia et al.). Owing to its high morbidity, high malignancy, high rate of recurrence after curable treatments, and resistance to traditional therapies, the 5-year survival rate of patients with untreated HCC is <5%, placing it among cancers with the worst prognosis (Parkin et al., 2005; Schütte et al., 2009). However, the prognosis can be obviously improved by early diagnosis, optimal treatment, and early detection of recurrence. For this purpose, serological tumor markers have been clinically used because of their convenience, inexpensiveness, and accuracy (Taketa et al., 1990; Fujiyama et al., 2002).

Alpha-fetoprotein (AFP) is the most widely investigated biomarker for diagnosing HCC. However, AFP has suboptimal diagnostic performance for HCC surveillance. First, increases in AFP levels are also observed in patients with chronic hepatitis and cirrhosis (Sterling et al., 2012; Bertino et al., 2009). Second, only a small proportion of early-stage HCCs (10–20%) present with elevated AFP levels (Yamashita et al., 2008). The EASL-EORTC (European Association for the Study of the et al., 2012) guidelines published in 2012 demonstrated that when combined with ultrasound (US), AFP levels can only improve the detection of previously identified cases by 6–8%. Thus, this recent guideline recommended against the use of AFP as a tumor marker in the flowcharts used for diagnosing HCC.

Since the 1980s, several novel tumor markers specific for HCC have been widely investigated, and numerous studies demonstrated that the Lens culinaris agglutinin-

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Table 1. Patients' Primary Characteristics (n= 53)

Variables	n (%)
Age (years), mean (range)	58 (37–77)
Sex	
Male	40 (75.47)
Female	13 (24.53)
Hepatitis virus infection	
Hepatitis B	37 (69.81)
Hepatitis C	14 (26.42)
Liver function	
Compensatory phase	14 (26.42)
Decompensatory phase	37 (69.81)
Child-Pugh classification	
A	41 (77.36)
B	10 (18.87)
C	2 (3.77)
ECOG score	
0	48 (90.57)
1	3 (5.66)
2	2 (3.77)
BCLC	
0	5 (9.43)
A	31 (58.49)
B	12 (22.65)
C	3 (5.66)
D	2 (3.77)

reactive fraction of AFP (AFP-L3) and Golgi protein 73 (GP73) were superior to AFP for the early diagnosis of HCC (Marrero et al., 2005; Durazo et al., 2008; Mao et al., 2010; Toyoda et al., 2011; Witjes et al., 2013), and they also could be used to monitor the response of patients to curative treatment and estimate the risk of relapse (Toyoda et al., 2008; Yamamoto et al., 2009; Mao et al., 2010; Nouso et al., 2011). However, most of these statistics were reported from Japan, and liver resection was the most commonly used curative treatment. Thus, whether these novel tumor markers could also be applied to evaluate treatment response and disease recurrence in Chinese patients with HCC after undergoing radiofrequency ablation (RFA) therapy are still unknown.

In this present study, we investigated the roles of AFP, AFP-L3, and GP73 in patients with HCC who underwent RFA therapy to determine whether these serum markers could be used as prognostic factors for monitoring short-term treatment response and detecting relapse after this kind of curative treatment.

Materials and Methods

Patients

Between July 2012 and July 2013, 53 consecutive patients with newly diagnosed HCC were enrolled and followed up prospectively at the First Hospital of Jilin University, Changchun, China. Among these 53 patients, 32 were scheduled to undergo RFA, and 24 patients completed 6-month follow-up visits. The patients' characteristics are presented in Table 1. This study was approved by the Institutional Ethnic Committee of Jilin University, and written informed consent was obtained from all the patients before enrollment.

The inclusion criteria for RFA of HCC were as follows:
1. Age of 18–70 years.
2. A solitary HCC tumor ≤ 7.0 cm in diameter, or multiple HCC lesions (≤3), each ≤3.0 cm in diameter.
3. HCC that was visible on US, with an acceptable/safe path between the tumor and the skin as shown on US.

The exclusion criteria were as follows:
1. Radiological evidence of invasion into the major portal/hepatic vein branches.
2. Patients with extrahepatic metastases, severe liver dysfunction (Child-Pugh class C/D), poor performance status (Eastern Cooperative Oncology Group (ECOG) Performance Status scale score, 3/4), or severe coagulation defects.

RFA technique

We used a commercially available system (RF 2000; Radio Therapeutics, Mounta in View, CA, USA) and a needle electrode with a 15-gauge insulated cannula with 10 hook-shaped expandable electrode tines with a diameter of 3.5 cm at expansion. After the 10 tines of the needle were deployed, the RF generator was activated and initiated with 10 W per minute of power, which was increased to 90 W per minute. RFA was applied until either a marked increase in impedance was noted or 15 min had elapsed. If a marked increase in impedance was not achieved, a second application of RF was given.

For tumors smaller than 3.0 cm, a single ablation was performed. For tumors larger than 3.0 cm, multiple overlapping ablations were performed. The first ablation started at the location farthest from the skin puncture site. After the ablation was completed, the electrode tines were retracted, and the needle was withdrawn to the second predetermined location. Then, the electrode tines were re-expanded, and the RF generator was reactivated. This process was repeated until the entire lesion was adequately covered.

Follow-up after RFA

Follow-up was conducted via an assessment of tumor markers (AFP, AFP-L3, and GP-73 levels) and dynamic computed tomography/magnetic resonance imaging at 1, 3, and 6 months post-RFA. The assessment of short-term response was based on the modified response evaluation criteria in solid tumors (mRECIST) (Llovet et al., 2008), according to the image results acquired 1 month after RFA. Local recurrence was considered to be present when new lesions were noted at, or adjacent to, the completely ablated lesion after RFA.

Tumor marker measurement

Blood samples for evaluation of the tumor markers were obtained 7 days before and 1 month after initiation of RFA therapy. Serum AFP levels were measured using an immunometric assay, and serum AFP-L3 levels were measured by lectin-affinity electrophoresis coupled with antibody-affinity blotting and expressed as the ratio of AFP-L3 to total AFP (%). Serum GP73 levels were measured using prototype enzyme-linked immunosorbent assays.

Statistical analysis

Correlations between the tumor marker values were

Table 2. Correlations of the Tumor-related Clinical Variables with the Tumor Marker Values

Clinical variables	AFP	AFP-L3	GP-73
Tumor number			
1	12.3 (5.85–84.74)	0.0 (0.0–20.07)	142 (87–196)
2	18.81 (8.33–56.10)	0.0 (0.0–27.81)	155 (129–189)
≥3	802.9 (74.82–1210)	16.90 (8.8–24.93)	213 (135–335)
	P = 0.002*	P = 0.233	P = 0.047
Vascular invasion			
No	22 (6.37–90.51)	0.0 (0.0–19.96)	144 (101.75–195.75)
Yes	1210 (93.5–42209.5)	15.9 (4.4–32.5)	157 (110–246.0)
	P = 0.015*	P = 0.257	P = 0.023*
Hepatitis virus infection			
hepatitis B	24.06 (6.01–159.45)	1.4 (0.0–23.455)	143 (94–196)
hepatitis C	29.14 (11.46–92.43)	0.18 (0.0–23.72)	192.5 (151.7–286.7)
	P = 0.866	P = 0.973	P = 0.011*
Child-Pugh classification			
1	20.27 (6.42–78.76)	0.0 (0.0–21.025)	141 (94–196)
2	83.62 (10.31–1052)	10.8 (0.0–18.07)	232.5 (153.5–351.25)
3	14683 (1210–28,156)	31.90 (27.81–36)	311 (285–337)
	P = 0.035*	P = 0.204	P = 0.016*
ECOG score			
0	19.54 (6.37–82.26)	0.0 (0.0–19.56)	144 (101.7–195.7)
1	1210 (1210–1210)	15.9 (0.8–27.81)	400 (337–400)
	P = 0.005*	P = 0.074	P = 0.007*
BCLC			
0	6.11 (3.14–76.47)	0.0 (0.0–10.035)	155 (74–257)
A	12.31 (5.91–56.1)	0.0 (0.0–16.4)	142 (90–185)
B	238.9 (30.08–1157.5)	17.23 (3.2–27.98)	170 (105.7–242.25)
C	175.3 (11.8–1210)	8.8 (0.0–36.0)	335 (157–400)
D	14683 (1210–28,156)	31.9 (27.81–36.0)	311 (285–337)
	P = 0.001*	P = 0.074	P = 0.049*

analyzed by Spearman's rank correlation (rs). Associations between the tumor marker values and the clinical variables were analyzed using Wilcoxon's rank sum test or the Kruskal-Wallis test, as appropriate. The changes in the marker values before and after RFA therapy were also analyzed by Wilcoxon's rank sum test. Associations between the marker values and the short-term treatment response and recurrence were evaluated by Fisher's exact test. $P < 0.05$ denoted statistical significance.

Results

Relationships between tumor markers

The AFP and AFP-L3 values displayed a close association (rs = 0.787, $p<0.001$), and the AFP and GP-73 values were mildly related (rs = 0.321, $p=0.023$). No significant correlation was found between the AFP-L3 and GP-73 levels (rs = 0.072, $p=0.608$).

Association between the tumor marker values and the clinical variables

The correlations of the AFP, AFP-L3, and GP-73 values with clinical variables are shown in Table 2. Increased AFP and GP-73 values were associated with the indices representing tumor growth and invasiveness such as tumor number, presence of vascular invasion, deterioration of liver function, advanced-stage disease, and poor performance status. Conversely, no apparent association was found between the AFP-L3 values and these indices.

Variations of the tumor marker values before and after RFA

The tumor marker values of the 32 patients before RFA and 1 month after RFA were compared. The AFP value was sharply decreased from 946.3 ng/mL to 19.04ng/mL ($P<0.001$), AFP-L3 value was dropped from 7.5% to 1.57% ($P=0.002$), and GP-73 was also declined from 154.44mAU/mL to 138.85mAU/mL ($P=0.035$).

Table 3. Association Between Tumor Marker Status Before RFA and 1 month after RFA with Short-term Outcome

Marker status before RFA		Short-term CR	Short-term PR	*P*
AFP	(–)	70.00% (7/10)	30.00% (3/10)	0.681
	(+)	77.27% (17/22)	22.73% (5/22)	
AFP-L3	(–)	71.43% (15/21)	28.57% (6/21)	0.681
	(+)	81.82% (9/11)	18.18% (2/11)	
GP-73	(–)	70.59% (12/17)	29.41% (5/17)	0.691
	(+)	80.00% (12/15)	20.00% (3/12)	
Marker status 1 month after RFA				
AFP	(–)	76.47% (13/17)	23.53% (4/17)	1
	(+)	73.33% (11/15)	26.67% (4/15)	
AFP-L3	(–)	76.67% (23/30)	23.33% (7/30)	0.444
	(+)	50.00% (1/2)	50.00% (1/2)	
GP-73	(–)	73.68% (14/19)	26.32% (5/19)	1
	(+)	76.92% (10/13)	23.08% (3/13)	

Table 4. Association Between Tumor Marker Status Before RFA and 1 month after RFA with Short-term Recurrence Rates

Marker status before RFA		CR within 6 months	Relapsed within 6 months	*P*
AFP	(–)	87.50% (7/8)	12.50% (1/8)	1
	(+)	81.25% (13/16)	18.75% (3/16)	
AFP-L3	(–)	87.50% (14/16)	12.50% (2/16)	0.578
	(+)	75.00% (6/8)	25.00% (2/8)	
GP-73	(–)	84.62% (11/13)	15.38% (2/13)	1
	(+)	81.82% (9/11)	18.18% (2/11)	
Marker status 1 month after RFA				
AFP	(–)	100.0% (13/13)	0.00% (0/13)	0.031*
	(+)	63.64% (7/11)	36.36% (4/11)	
AFP-L3	(–)	86.36% (19/22)	13.64% (3/22)	0.312
	(+)	50.00% (1/2)	18.18% (1/2)	
GP-73	(–)	85.71% (12/14)	14.29% (2/14)	1
	(+)	80.00% (8/10)	20.00% (2/10)	

Prognostic values on short-term response

From these 53 patients, 32 were scheduled to undergo RFA, and short-term response was evaluated 1 month after RFA. According to the mRECIST, 24 patients achieved complete remission, and eight patients displayed partial remission. In this analysis, neither the pre-RFA nor the 1 month post-RFA tumor marker status was associated with short-term outcomes (Tables 3).

Prognostic value of AFP, AFP-L3, and GP-73 for short-term recurrence

Among the 32 patients who underwent RFA, 24 completed 6-month follow-up visits, among whom four patients experienced a relapse within 6 months. The AFP, AFP-L3, and GP-73 statuses before treatment were not associated with short-term recurrence. The short-term recurrence rate of the AFP-positive patients measured 1 month after RFA was obviously higher than that of the AFP-negative patients ($P=0.031$) (Table 4).

Discussion

AFP can be fractionated by affinity electrophoresis into three glycoforms, namely L1, L2, and L3, based on its reactivity with the lectin Lens culinaris agglutinin. The L1 isoform is typically associated with benign liver disease, and the L3 isoform is specific to malignant HCC (Yoshida et al., 2002). AFP-L3 is an isoform of AFP, and it is clinically reported as the percentage of AFP-L3 to total AFP; thus, the AFP-L3 value is associated with AFP. GP73 was originally described as a resident Golgi type II transmembrane protein expressed primarily in epithelial cells of many human tissues. GP73 antigen expression is barely detectable in healthy subjects, but it is elevated modestly in virus carriers, moderately in patients with cirrhosis, and dramatically in patients with HCC (Block et al., 2005). Mao et al (Mao et al., 2010) compared serum GP73 and AFP levels in 4217 human subjects in a multicenter study in 2010, finding that the sensitivity and specificity of GP73 level for the detection of HCC were 74.6% and 97.4%, respectively, significantly higher than the corresponding values for AFP level. Iman et al. (2013) recently reported that in Egyptian patients the sensitivity and specificity of serum GP 73 for early detection of HCC were 95% each, thus GP 73 was a promising diagnostic marker. A recent meta-analysis (Witjes et al., 2013) also indicated that GP73 level was superior to AFP level for the early diagnosis and screening of HCC. In our study, we identified GP-73 as an independent tumor marker that is not associated with AFP and AFP-L3. Although AFP-L3 was associated with AFP, many previous studies demonstrated that AFP-L3 was a better tumor marker for the early diagnosis of HCC than AFP. Thus, we can combine these three tumor markers in clinical practice to improve the early detection of HCC.

HCC biomarkers have also been reported to be predictive of specific clinicopathological variables representing the malignant potential of the tumor. Many studies (Tangkijvanich et al., 2000; Fujioka et al., 2001; Carr et al., 2007; Yamamoto et al., 2010; Saito et al., 2012) revealed that AFP levels > 400 ng/mL were indicative of larger tumor size, greater tumor numbers, a later clinical phase, bile duct invasion, vascular invasion, and a shorter median survival time. Elevated AFP-L3 levels were associated with larger tumor size, a later clinical stage, vascular invasion, poor tumor differentiation, and distant metastasis (Oka et al., 2001; Yoshida et al., 2002; Carr et al., 2007; Saito et al., 2012). Other research studies (Riener et al., 2009; Hu et al., 2010) observed that GP-73 levels were significantly higher in patients with hepatitis C-derived HCC and a high tumor grade. In our study, we evaluated tumor size, tumor number, and vascular invasion by imaging conducted before RFA and collected clinical information about hepatitis infection status, liver function, clinical stage, and other variables. We analyzed the association between these clinical variables and serum tumor marker levels and found that increased AFP and GP-73 levels were associated with variables representing tumor growth and invasiveness such as tumor number, the presence of vascular invasion, deteriorated liver function, advanced stage, and poor performance status. Although the mechanism was unclear, our study also demonstrated that GP-73 levels were dramatically elevated in the patients with hepatitis C-derived HCC. Owing to some limitations of our study, no correlation was found between the AFP-L3 levels and these clinical variables. First, all of these variables were obtained from imaging analysis, as opposed to surgical specimens. Second, the sample size of our study was small; therefore, positive results may be obtained with larger sample sizes.

To date, the curative treatments of HCC include surgical resection, liver transplantation, and RFA. A five-year survival rate of 70% and preserved hepatic function after the surgical resection of single tumors less than 5 cm in diameter have been achieved in patients with HCC. In addition, 5-year survival rates exceeding 70% have been reported in patients with HCC meeting the Milan criteria (single nodule < 5 cm or three nodules each < 3 cm in diameter) after liver transplantation. Moreover, if patients with HCC who were not candidates for surgical resection or liver transplantation underwent RFA, their 5-year overall survival rates could be improved to 37% (Llovet and Bruix 2000; Ioannou et al., 2008). Xin Dai et al (Dai et al., 2012) recently demonstrated that RFA also had the advantages of accurate localization, good efficacy, easy operation, and minimal invasion without any complications in the treatment of HCC recurrence after liver transplantation. In our study AFP, AFP-L3, and GP-73 levels all sharply decreased after RFA, which indicated that these tumor markers could reflect the tumor burden and demonstrate the efficacy of RFA.

In this analysis, neither the pre-RFA nor the 1-month post-RFA tumor marker values were associated with short-term outcomes. Thus, in clinical practice, we should give priority to imaging data instead of serum tumor marker measurements for evaluating treatment response 1 month after RFA.

Tateishi et al. (2006) conducted a study to elucidate the accuracy of tumor markers in predicting recurrence after a curative ablation of HCC. Multivariate analysis indicated that AFP levels > 100 ng/mL and AFP-L3 values > 15% both preablation and postablation were significant predictors of recurrence. AFP-L3 preablation was a significant predictor of recurrence in the multivariate analysis, but it retained no significance in the patients whose AFP-L3 value had decreased to less than 15% after ablation. This finding may indicate that ablation therapy is highly effective even for poorly differentiated HCC, and the poor prognosis of patients with AFP-L3-positive HCC may be reversible if complete ablation of the tumor results in AFP-L3 negativity. In addition, a recent study in Japan (Tamura et al., 2013) also indicated that AFP-L3 status 1 month after treatment was a significant independent predictor of HCC recurrence after curative treatment. In this analysis, we had a similar conclusion that the short-term recurrence rate of the AFP-positive patients 1 month post-RFA was obviously higher than that of AFP-negative patients.

In conclusion, AFP and GP-73 levels were associated with clinical variables representing tumor growth and invasiveness, and we should focus on the pathological variables in surgical specimens in further research. AFP

value is a strong predictor of HCC short-term recurrence, and we need to increase the sample size and extend the follow-up time in future research.

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