

# Alpha-Fetoprotein Has No Prognostic Role in Small Hepatocellular Carcinoma Identified During Surveillance in Compensated Cirrhosis

Edoardo G. Giannini,<sup>1</sup> Simona Marengo,<sup>1</sup> Giacomo Borgonovo,<sup>1</sup> Vincenzo Savarino,<sup>1</sup> Fabio Farinati,<sup>2</sup> Paolo Del Poggio,<sup>3</sup> Gian Ludovico Rapaccini,<sup>4</sup> Maria Anna Di Nolfo,<sup>5</sup> Luisa Benvegnù,<sup>6</sup> Marco Zoli,<sup>7</sup> Franco Borzio,<sup>8</sup> Eugenio Caturelli,<sup>9</sup> Maria Chiaramonte,<sup>10</sup> and Franco Trevisani<sup>11</sup> for the Italian Liver Cancer (ITA.LI.CA) group

**Alpha-fetoprotein is a tumor marker that has been used for surveillance and diagnosis of hepatocellular carcinoma (HCC) in patients with cirrhosis. The prognostic capability of this marker in patients with HCC has not been clearly defined. In this study our aim was to evaluate the prognostic usefulness of serum alpha-fetoprotein in patients with well-compensated cirrhosis, optimal performance status, and small HCC identified during periodic surveillance ultrasound who were treated with curative intent. Among the 3,027 patients included in the Italian Liver Cancer study group database, we selected 205 Child-Pugh class A and Eastern Cooperative Group Performance Status 0 patients with cirrhosis with a single HCC  $\leq 3$  cm of diameter diagnosed during surveillance who were treated with curative intent (hepatic resection, liver transplantation, percutaneous ethanol injection, radiofrequency thermal ablation). Patients were subdivided according to alpha-fetoprotein serum levels (i.e., normal  $\leq 20$  ng/mL; mildly elevated 21-200 ng/mL; markedly elevated  $>200$  ng/mL). Patient survival, as assessed by the Kaplan-Meier method, was not significantly different among the three alpha-fetoprotein classes ( $P = 0.493$ ). The same result was obtained in the subgroup of patients with a single HCC  $\leq 2$  cm ( $P = 0.714$ ). An alpha-fetoprotein serum level of 100 ng/mL identified by receiver operating characteristic curve had inadequate accuracy (area under the curve = 0.536, 95% confidence interval = 0.465-0.606) to discriminate between survivors and deceased patients. **Conclusion:** Alpha-fetoprotein serum levels have no prognostic meaning in well-compensated cirrhosis patients with single, small HCC treated with curative intent. (HEPATOLOGY 2012;56:1371-1379)**

**H**epatocellular carcinoma (HCC) is the third cause of cancer death and the leading cause of mortality among patients with cirrhosis.<sup>1</sup> Liver cirrhosis is in fact the main risk factor for HCC, and the annual incidence of HCC in cirrhosis patients is 3%-7%.<sup>2,3</sup>

Detecting HCC at an early stage is the main objective of screening and surveillance programs.<sup>3</sup> Indeed, the utility of surveillance for HCC in patients with

cirrhosis is supported by the results of a randomized trial carried out in patients with chronic hepatitis B virus (HBV) infection and several cohort studies performed in patients with cirrhosis.<sup>4-7</sup> Surveillance of patients at risk of HCC with liver ultrasound, with or without serum alpha-fetoprotein assessment, is recommended by European, American, and Asiatic HCC management guidelines with the aim to identify HCC at an early stage in those patients who, in the event of

*Abbreviations:* AASLD, American Association for the Study of Liver Diseases; BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ITA.LI.CA, Italian Liver Cancer; PEI, percutaneous ethanol injection; RFTA, radiofrequency thermal ablation; ROC, receiver operating characteristic.

From the <sup>1</sup>Dipartimento di Medicina Interna, Unità di Gastroenterologia, Università di Genova, Italy; <sup>2</sup>Dipartimento di Scienze Chirurgiche e Gastroenterologiche, Unità di Gastroenterologia, Università di Padova, Italy; <sup>3</sup>Divisione di Medicina, Ospedale Treviglio-Caravaggio, Treviglio, Italy; <sup>4</sup>Unità di Medicina Interna e Gastroenterologia, Università Cattolica di Roma, Roma, Italy; <sup>5</sup>Divisione di Medicina, Azienda Ospedaliera Bolognini, Seriate, Italy; <sup>6</sup>Dipartimento di Medicina Clinica e Sperimentale, Unità di Medicina, Università di Padova, Italy; <sup>7</sup>Dipartimento di Medicina Interna, dell'Invecchiamento e Malattie Nefrologiche, Unità di Medicina Interna, Alma Mater Studiorum – Università di Bologna, Italy; <sup>8</sup>Dipartimento di Medicina, Unità di Medicina Interna ed Epatologia, Ospedale Fatebenefratelli, Milano, Italy; <sup>9</sup>Unità di Gastroenterologia, Ospedale Belcolle, Viterbo, Italy; <sup>10</sup>Unità di Gastroenterologia, Ospedale Sacro Cuore Don Calabria, Negrar, Italy; <sup>11</sup>Dipartimento di Medicina Clinica, Unità di Semeiotica Medica, Alma Mater Studiorum – Università di Bologna, Italy.

Received February 24, 2012; accepted April 22, 2012.

cancer detection, are amenable to curative therapies able to improve their prognosis.<sup>8-10</sup> The American Association for the Study of Liver Diseases (AASLD) guidelines for HCC diagnosis and treatment, however, recently dropped alpha-fetoprotein assessment from the surveillance armamentarium due to poor sensitivity for early diagnosis of HCC and unacceptable specificity of this tumoral marker.<sup>10</sup> Nonetheless, the use of alpha-fetoprotein as a prognostic indicator when HCC is diagnosed in the most favorable setting—patients with compensated cirrhosis, optimal performance status, single, small HCC, and as such amenable to curative treatment—has not been sufficiently addressed so far.<sup>11</sup> In particular, some studies that evaluated the prognostic usefulness of alpha-fetoprotein in patients with HCC suggested that its prognostic role may be influenced by size and number of the HCC nodules, although contrasting results have been obtained.<sup>11-14</sup>

Single, small HCC in patients with compensated cirrhosis and optimal performance status are identified by the Barcelona Clinic Liver Cancer (BCLC) classification as very early (class 0) and early (class A) HCC, and at this stage patients are usually amenable to curative treatment.<sup>15</sup> The BCLC classification system, however, does not include alpha-fetoprotein assessment, although this serum marker has been identified by several studies as an overall independent predictor of survival.<sup>11</sup> However, the majority of studies that evaluated the prognostic capability of alpha-fetoprotein have included heterogeneous cohorts of patients, thus preventing an appropriate assessment of its usefulness as a prognostic tool in a well-defined subset of patients.<sup>11,16</sup>

In this study we evaluated the prognostic role of alpha-fetoprotein in patients with compensated cirrhosis, optimal performance status, and single, small HCC ( $\leq 3$  cm) identified during surveillance and treated with curative intent. Our aim was to verify whether, in this specific setting, assessment of alpha-fetoprotein serum levels may have any prognostic relevance.

## Patients and Methods

**Patients.** We retrospectively analyzed the data of the Italian Liver Cancer (ITA.LI.CA) database, cur-

rently including 3,027 HCC patients consecutively seen from January 1987 to December 2008 at 11 Italian medical institutions. The data were collected prospectively and updated every 2 years. Main characteristics of the database have been previously reported.<sup>17</sup> Briefly, the ITA.LI.CA database includes data on patient demographics, main biochemical and hematological variables, etiology and stage of liver disease, presence of comorbidities, HCC stage and treatment, patient survival, and causes of death.<sup>17</sup>

For the purpose of this study, we included patients with well-compensated liver cirrhosis (Child-Pugh class A) and Eastern Cooperative Oncology Group Performance Status of 0 who were diagnosed with a single, small (i.e.,  $\leq 3$  cm) HCC during periodic liver ultrasound, had no vascular invasion, no metastases, and who were treated with curative intent.<sup>18,19</sup>

**Diagnosis, Staging, and Treatment of HCC.** The diagnosis of HCC was based on histology and/or cytology in 106 (51.7%) patients. In the remainder, diagnosis was confirmed by combining an alpha-fetoprotein value  $>200$  ng/mL with typical features in one imaging technique (dynamic computed tomography, magnetic resonance imaging, or contrast-enhanced ultrasound) or, in the absence an alpha-fetoprotein value  $>200$  ng/mL, in at least two imaging techniques.<sup>8</sup> Tumor size (maximum diameter, expressed in cm) was assessed on imaging.

When available, in patients in whom the diagnosis of HCC was histologically confirmed by fine-needle aspiration biopsy, surgical specimen, or explanted liver, the tumor was graded according to the Edmondson and Steiner classification.<sup>20</sup> For consistency, we grouped grades I and II (well and moderately differentiated) and grades III and IV (poorly differentiated) tumors.<sup>21</sup>

This study included patients who were treated with curative intent alone, considering curative the surgical (orthotopic liver transplantation, hepatic resection) and percutaneous ablative (percutaneous ethanol injection [PEI] or radiofrequency thermal ablation [RFTA]) techniques.

**Alpha-Fetoprotein and Survival.** Alpha-fetoprotein was determined at the time of HCC diagnosis. Alpha-fetoprotein levels were classified as normal ( $\leq 20$  ng/mL), mildly elevated (21-200 ng/mL), and markedly

Address reprint requests to: Edoardo G. Giannini, M.D., Ph.D., F.A.C.G., Gastroenterology Unit, Department of Internal Medicine, University of Genoa, Viale Benedetto XV, no. 6, 16132, Genoa, Italy. E-mail: egiannini@unige.it; fax: +39 010 353 8638.

Copyright © 2012 by the American Association for the Study of Liver Diseases.

View this article online at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).

DOI 10.1002/hep.25814

Potential conflict of interest: Nothing to report.

**Table 1. Main Demographic, Biochemical, and Clinical Characteristics of the 205 Study Patients**

Variable	Unit	Value
Age	years	69 (46-89)
Gender	male	135 (65.8)
ALT	n x ULN	1.8 (0.5-7.5)
Albumin	g/dL	3.9 (3.2-4.8)
Total bilirubin	mg/dL	1.0 (0.3-2.1)
Prothrombin activity	(%)	81 (61-110)
Platelet count	x10 <sup>9</sup> /L	115 (61-363)
Alpha-fetoprotein	ng/mL	14.0 (1.0-4,185)
HCC diameter	cm	2.0 (0.8-3.0)
Esophageal varices*	present	53 (32.5)

Data are shown as median value (range) or absolute frequency (%).

\*Data regarding esophageal varices were available in 163 patients (79.5). ULN, upper limit of normal; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma.

elevated (>200 ng/mL). Overall survival was calculated from the time of HCC diagnosis to death or to December 2008. Patients lost to follow-up (n = 22, 10.7%) were censored at the time of the last clinical examination.

**Statistical Analysis.** Continuous data are expressed as median value and range, and discrete variables as absolute and relative frequencies. To compare continuous variables we applied the Mann-Whitney *U* test and the Kruskal-Wallis test, whereas discrete variables were compared with the  $\chi^2$  test with Yates' correction and Fisher's exact test, as appropriate. Patient survival was assessed according to the Kaplan-Meier method and compared by the log-rank test. A receiver operating characteristic (ROC) curve was used to identify the alpha-fetoprotein value with the highest accuracy for discriminating between survivors and deceased patients. Moreover, the ROC curve was used to identify the cutoff prevalence-adjusted positive and negative predictive values, and positive and negative likelihood ratios for death. A 2-tailed *P*-value < 0.05 was considered statistically significant. Statistical analysis was performed using MedCalc statistical package (MedCalc Software, Mariakerke, Belgium).

**Ethics.** The ITA.LI.CA database management conforms to the past and current Italian legislation on the privacy and the present study conforms to the ethical guidelines of the Declaration of Helsinki. Approval for the study was obtained by the Institutional Review Board of the participating centers.

**Results**

The main demographic, biochemical, and clinical characteristics of the 205 study patients are reported in Table 1. The main cause of liver cirrhosis was chronic

infection with hepatitis viruses (n = 180, 87.8%). The Child-Pugh score was 5 in 151 patients (73.7%), and the maximum diameter of the HCC nodule was ≤2 cm in 122 patients (59.5%). Serum alpha-fetoprotein levels were within the normal range (≤20 ng/mL) in 116 patients (56.6%), mildly elevated (21-200 ng/mL) in 71 patients (34.6%), and markedly elevated (>200 ng/mL) in 18 patients (8.8%). Seven patients (3.4%) had an extremely high (>400 ng/mL) serum alpha-fetoprotein level. Treatment of HCC was liver transplantation in three patients (1.5%), hepatic resection in 53 patients (25.8%), RFTA and PEI in 66 (32.2%) and 83 (40.5%) patients, respectively. Median duration of follow-up was 3.7 years, and median time to death was 48 months. Figure 1 shows the log-transformed serum alpha-fetoprotein levels in the 205 patients subdivided according to status (survivors and deceased).

Among the 180 patients with viral etiology of liver disease, 154 patients (85.6%) had chronic hepatitis C virus (HCV) infection (including four patients with chronic HCV-HBV coinfection), and 26 patients (14.4%) had chronic HBV infection alone. All in all, 47 patients had been treated with interferon-based antiviral therapy before HCC diagnosis (41 HCV-positive alone, 4 HBV-positive alone, and 2 with HCV-HBV coinfection). Among HBV patients, seven were being treated with nucleos(t)ide analogs at the time of HCC diagnosis. We subdivided viral patients into two groups—those with current and past antiviral therapy (n = 50) versus those who received no antiviral therapy at all (n = 104)—and evaluated alpha-fetoprotein levels in these two groups. The median alpha-fetoprotein level was 19.5 ng/mL (range, 2.0-4,185 ng/mL) and 16.0 ng/mL (range, 1.0-1,600 ng/mL), respectively (*P* = 0.874).

F1

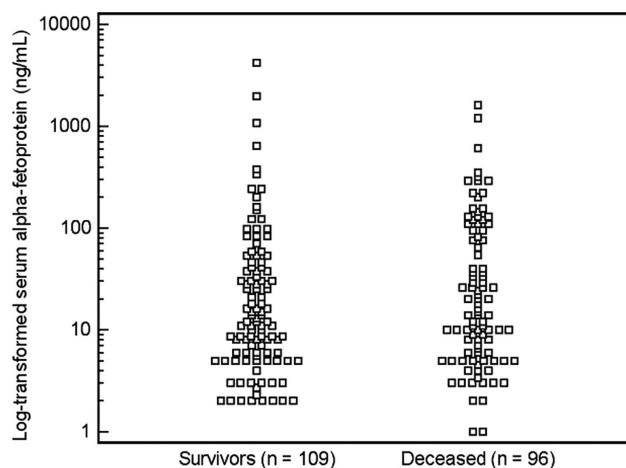


Fig. 1. Plot of log-transformed serum alpha-fetoprotein levels of all study patients (n = 205) subdivided according to status (survivors and deceased).

T1

**Table 2. Main Demographic, Biochemical, and Clinical Characteristics of the 205 Study Patients Subdivided According to Serum Alpha-Fetoprotein Levels**

Variable	Unit	Serum Alpha-Fetoprotein Level			P-value
		0-20 (n = 116)	21-200 (n = 71)	>200 (n = 18)	
Age	years	69 (47-89)	69 (46-84)	69 (58-84)	0.953
Gender	male	87 (75.0)	39 (54.9)	9 (50.0)	0.007
ALT	n x ULN	1.5 (0.4-6.6)	2.0 (0.3-7.5)	1.8 (0.9-5.0)	0.011
Child-Pugh	score 5	84 (72.4)	52 (73.2)	15 (83.3)	0.616
HCC diameter	cm	2.0 (0.8-3.0)	2.0 (0.8-3.0)	2.4 (1.0-3.0)	0.163
HCC treatment	Resection	32 (27.6)	15 (21.1)	6 (33.3)	0.444
	OLT	0 (0)	2 (2.8)	1 (5.6)	
	PEI	49 (42.2)	29 (40.9)	5 (27.8)	
	RFTA	35 (30.2)	25 (35.2)	6 (33.3)	
Deceased	n	52 (44.8)	34 (47.9)	10 (55.6)	0.681
Cause of death	HCC progression	28 (53.8)	16 (47.1)	5 (50.0)	0.825
	Liver failure	10 (19.2)	8 (23.5)	2 (20.0)	
	Bleeding	5 (9.6)	3 (8.8)	1 (10.0)	
	Sepsis	2 (3.8)	1 (2.9)	1 (10.0)	
	Other	5 (9.6)	5 (14.7)	1 (10.0)	
	Not available	2 (3.8)	1 (2.9)	0 (0)	

Data are shown as median value (range) or absolute frequency (%).

ALT, alanine aminotransferase; ULN, upper limit of normal; HCC, hepatocellular carcinoma; OLT, orthotopic liver transplantation; PEI, percutaneous ethanol injection; RFTA, radiofrequency thermal ablation.

T2 Table 2 shows the main demographic and clinical characteristics of the patients subdivided according to alpha-fetoprotein levels ( $\leq 20$  ng/mL; 21-200 ng/mL;  $> 200$  ng/mL). Among the parameters evaluated, female gender ( $P = 0.007$ ) and greater increase in alanine aminotransferase ( $P = 0.011$ ) were significantly more common in patients with mildly (21-200 ng/mL) or markedly elevated ( $> 200$  ng/mL) alpha-fetoprotein levels. HCC diameter and degree of liver failure were not significantly different among the three alpha-fetoprotein classes. Modality of HCC treatment (surgical versus ablation,  $P = 0.444$ ) and causes of death were similar among the three groups. Edmondson grading was available only in a minority of patients in all classes (27% in patients with alpha-fetoprotein  $\leq 20$  ng/mL; 17% in patients with alpha-fetoprotein 21-200 ng/mL; 11% in patients with alpha-fetoprotein  $> 200$  ng/mL). Despite this limitation, patients with well and moderately differentiated HCCs tended to be more frequently observed in the group with normal ( $\leq 20$  ng/mL) or mildly elevated (21-200 ng/mL) alpha-fetoprotein levels ( $P = 0.056$ ).

During the follow-up, 96 patients (46.8%) died and the proportion of deceased patients was similar in the three alpha-fetoprotein classes ( $\leq 20$  ng/mL; 21-200 ng/mL;  $> 200$  ng/mL). Similarly, the causes of death were not different across the three alpha-fetoprotein

F2 classes. Figure 2 shows the actuarial survival curves of these patients. There was no statistically significant difference among the three alpha-fetoprotein classes ( $\chi^2 = 1.4162$ ,  $P = 0.493$ ). The analysis was repeated in

the subgroup of 122 patients with a nodule  $\leq 2$  cm (Fig. 3A) and in the subgroup of 83 patients with a nodule 2-3 cm (Fig. 3B): again, no significant survival difference was observed among the three alpha-fetoprotein classes (HCC  $\leq 2$  cm:  $\chi^2 = 0.6744$ ,  $P = 0.714$ ; HCC 2-3 cm:  $\chi^2 = 2.0926$ ,  $P = 0.351$ ). We also compared survival between patients with normal ( $\leq 20$  ng/mL) and elevated ( $> 20$  ng/mL) alpha-fetoprotein (Fig. 4A), and between patients with an alpha-fetoprotein above or below 200 ng/mL (Fig. 4B). Even with these cutoffs, no statistically significant differences

F3

F4

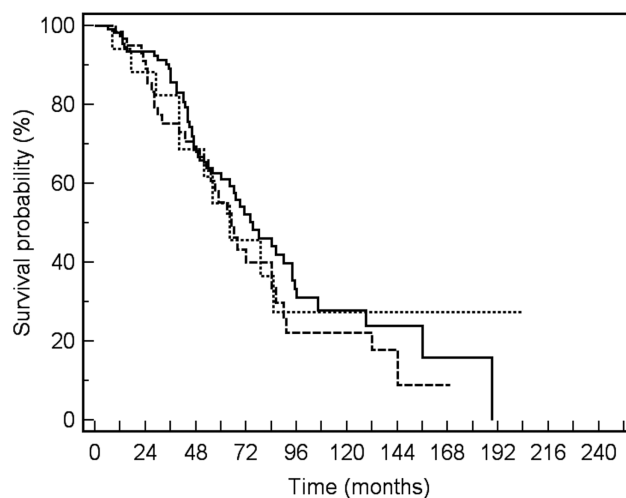


Fig. 2. Kaplan-Meier survival curves of the 205 study patients subdivided according to their alpha-fetoprotein serum levels at the diagnosis of hepatocellular carcinoma (solid line,  $\leq 20$  ng/mL; dashed line, 21-200 ng/mL; dotted line  $> 200$  ng/mL).

F5

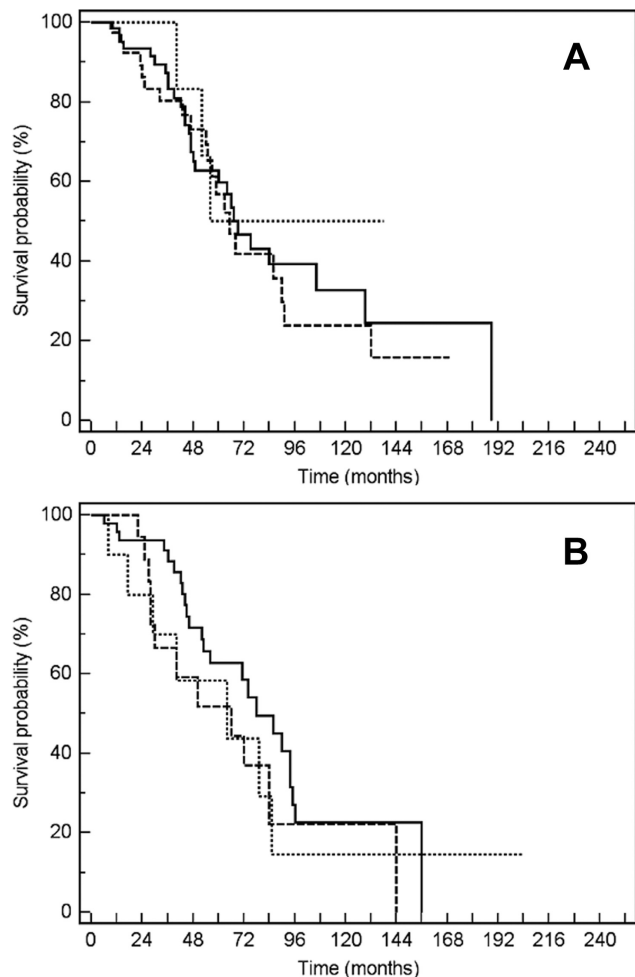


Fig. 3. Kaplan-Meier survival curves of the 122 study patients with HCC  $\leq 2$  cm (A) and of the 83 study patients with HCC between 2 and 3 cm (B), subdivided according to their alpha-fetoprotein serum levels (solid line,  $\leq 20$  ng/mL; dashed line, 21-200 ng/mL; dotted line  $> 200$  ng/mL).

were observed. Lastly, we evaluated treatment and survival of the seven patients with extremely high alpha-fetoprotein levels ( $> 400$  ng/mL): three (42.9%) had a tumor  $\leq 2$  cm, four underwent hepatic resection, and three percutaneous ablation. Four patients died after a median of 56 months (range, 17-79 months) and three were alive after a median of 60 months (range, 6-100 months). Taking into account the caveat such an analysis may bear, due to the very small sample size, there was no survival difference between patients with alpha-fetoprotein above and below 400 ng/mL ( $\chi^2 = 0.137$ ,  $P = 0.712$ ).

The ROC curve showed that alpha-fetoprotein had inadequate accuracy to discriminate survivors and deceased patients (area under the ROC curve = 0.536, 95% confidence interval [CI] = 0.465-0.606). A ROC curve-identified alpha-fetoprotein cutoff of 100 ng/mL had good specificity (88%, 95% CI = 81%-94%) but

unacceptably low sensitivity (23%, 95% CI = 15%-33%) for discriminating survivors and deceased patients (Fig. 5). Prevalence-adjusted positive and negative predictive values for death of this cutoff were 63.6% and 56.5%, respectively, whereas positive and negative likelihood ratios were 1.96 and 0.86, respectively. Moreover, there was no significant survival difference between patients with an alpha-fetoprotein below or above this cutoff ( $\chi^2 = 0.8301$ ;  $P = 0.367$ ).

Lastly, we also evaluated the predictors of death in this very homogenous population of cirrhosis patients with HCC and found that the type of curative treatment (hepatic surgery, median survival 86 months versus ablative treatment, median survival 64 months,  $P = 0.019$ ) was the only predictor of survival, whereas there was no significant survival difference associated with gender, age below 65 years, etiology of liver disease (viral versus nonviral), presence of esophageal

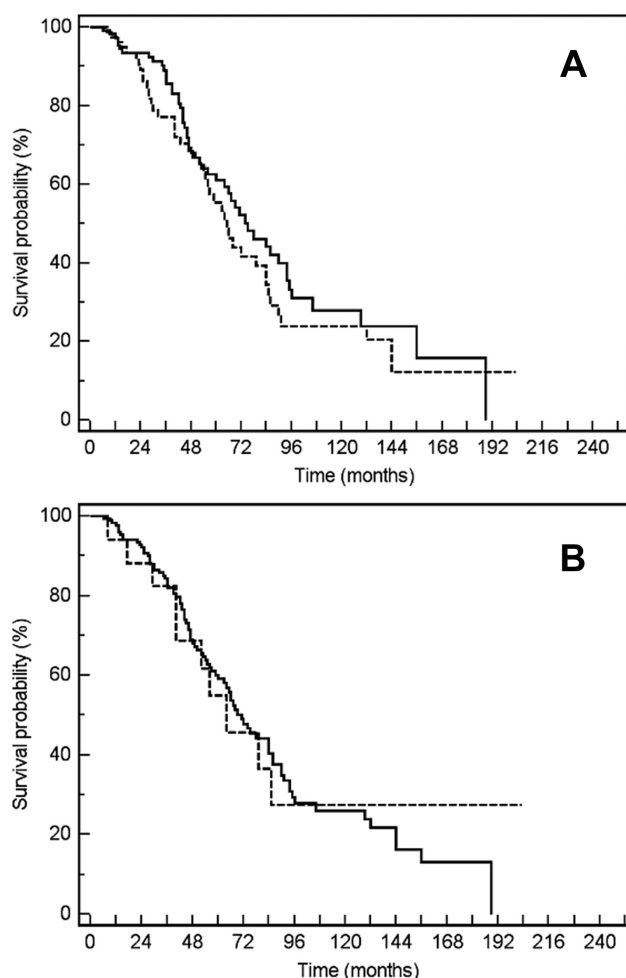


Fig. 4. Kaplan-Meier survival curves of the 205 study patients subdivided according to their alpha-fetoprotein serum levels at the diagnosis of hepatocellular carcinoma (A: solid line,  $\leq 20$  ng/mL; dashed line,  $> 20$  ng/mL; B: solid line,  $\leq 200$  ng/mL; dashed line,  $> 200$  ng/mL).

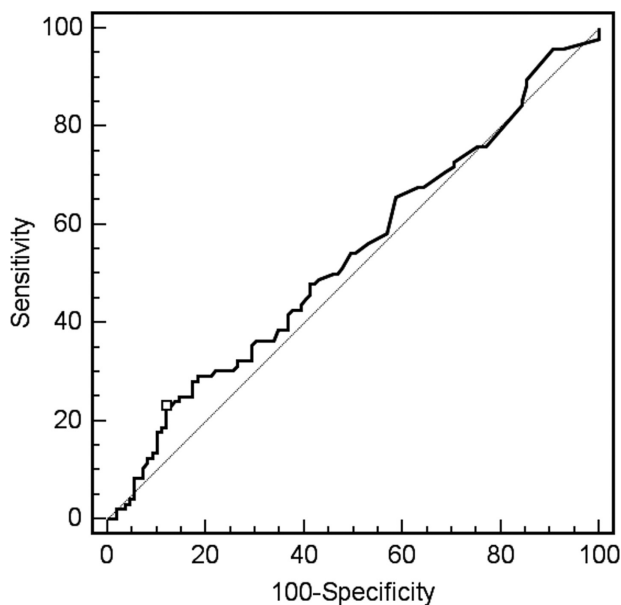


Fig. 5. ROC curve showing the overall accuracy of alpha-fetoprotein serum levels for discriminating between survivors and deceased patients. The empty dot identifies the best cutoff value (i.e., 100 ng/mL) of serum alpha-fetoprotein.

varices (datum available in 163 patients), and size of HCC ( $\leq 2$  or 2-3 cm).

## Discussion

The usefulness of serum alpha-fetoprotein as a surveillance and diagnostic test for HCC has been dramatically challenged by the impressive technical improvement of abdominal ultrasound and contrast medium-enhanced diagnostic imaging that have led to great accuracy in the early identification and noninvasive characterization of small HCCs.<sup>22</sup> In fact, it has repeatedly been shown that serum alpha-fetoprotein levels tend to be nonspecifically altered in patients affected by chronic liver disease, and that diagnostic levels of this tumor marker are seldom observed in patients with small HCCs.<sup>23-27</sup> This evidence has led the providers of the updated AASLD guidelines for the diagnosis and management of HCC to drop alpha-fetoprotein from the surveillance armamentarium for HCC in patients with cirrhosis, although this decision was debated.<sup>10,28,29</sup>

However, alpha-fetoprotein may have a prognostic meaning in patients with HCC, and is included in prognostic classifications such as the Cancer of the Liver Italian Program score, although also in this setting the results of various studies have provided inconsistent findings.<sup>11-14,30,31</sup> Recently, Tandon and Garcia-Tsao<sup>11</sup> performed a comprehensive, systematic review of prognostic indicators in HCC and identified

alpha-fetoprotein as one of the most robust prognostic indexes, although they observed that the appropriate cutoff level and group of patients in which this serum marker may be helpful remain to be established. Thus, we deemed it of interest to evaluate whether alpha-fetoprotein might be a prognostic indicator in patients who might benefit most from the application of curative treatment, and therefore where prognostication should be of utmost importance.

In this study we demonstrated that alpha-fetoprotein has no prognostic relevance in patients with well-compensated cirrhosis, optimal performance status, and a single, small HCC (i.e.,  $\leq 3$  cm) identified during surveillance and treated with curative intent. The poor prognostic performance of alpha-fetoprotein we observed in this particular setting may be due to several reasons. First, alpha-fetoprotein levels were within the normal range in more than half of the population, and reached markedly elevated levels (i.e.,  $>200$  ng/mL) in less than 10% of the patients. These findings are strikingly in keeping with previous features (i.e., 11.1%) observed in a population of 153 patients with small ( $<2$  cm) HCC seen in our geographical area.<sup>32</sup> Even when patients were more broadly subdivided according to normal or elevated alpha-fetoprotein levels (i.e., above or below 20 ng/mL) no survival difference surfaced between the two groups. Furthermore, in order to avoid limitations related to the use of pretest fixed cutoffs of a continuous variable, we also performed an analysis using an *ad hoc* alpha-fetoprotein cutoff identified by ROC curve. However, even this analysis showed that alpha-fetoprotein had negligible prognostic accuracy (area under the ROC curve = 0.536, 95% CI = 0.465-0.606), and the ROC curve-identified alpha-fetoprotein cutoff (i.e., 100 ng/mL) had largely inadequate sensitivity (23%, 95% CI = 15%-33%). Second, as previously reported by others,<sup>33,34</sup> we too observed that increased alpha-fetoprotein levels were associated with female gender and greater hepatic cytolytic activity, although they had no association with clinical and tumoral characteristics, and were not influenced by current and past antiviral therapy. In this regard, it has to be emphasized that some of its determinants such as degree of liver failure and HCC size varied within a narrow range as per study inclusion criteria. These findings confirm that the prognostic role of alpha-fetoprotein reported in other studies may be due to the heterogeneous liver- and tumor-related characteristics, and different modalities of HCC treatment in the studied populations.<sup>11,16</sup> In fact, it seems that the predictive ability of alpha-fetoprotein is highly dependent on tumor size

and treatment strategy, being more apparent in patients with advanced HCC and in those treated with palliative intention, and less evident in patients with small tumors and in those who underwent curative treatment.<sup>11-14,30,35</sup> Indeed, in studies where patients with advanced liver disease and/or advanced HCC were excluded from the analyses, the prognostic role of alpha-fetoprotein was dramatically diluted.<sup>12,30</sup> These considerations are also supported by the evidence that in our series there was no “therapeutic disparity,” and that mortality and causes of death were evenly distributed across patients with normal, mildly, and markedly elevated alpha-fetoprotein levels, likely ruling out the presence of other possible prognostic confounding factors.

Some studies have shown that the rate of rise of serum alpha-fetoprotein levels may have prognostic meaning in HCC patients awaiting liver transplantation, yet these studies did not identify static alpha-fetoprotein levels as a predictor of survival or HCC recurrence after liver transplantation.<sup>36-38</sup> As serial alpha-fetoprotein determinations were not available in our patients, we were not able to assess the possible prognostic role of dynamic alpha-fetoprotein determinations in the clinical setting of this study.

In this study we selected the 3-cm cutoff to define small HCC, as several studies have shown an excellent outcome after curative treatment in these patients, and this threshold is also accepted for curative treatment by the Asian Pacific Association for the Study of the Liver.<sup>9,39,40</sup> However, we also performed the same analyses in patients with an HCC  $\leq 2$  cm, as other studies have shown that the prevalence of the two main negative prognostic factors, microvascular invasion and satellite nodules, tends to increase in lesions above this threshold.<sup>41-43</sup> We confirmed, also in this group of HCC classified “very early” (stage 0) by the BCLC system, that serum alpha-fetoprotein had no prognostic role, thus confuting the hypothesis that adding this tumor marker to the BCLC classification might increase its prognostic yield for patients with very early (stage 0) and early (stage A) HCCs.<sup>11,16</sup>

Noteworthy, in our cohort the 5-year survival rate was  $\approx 60\%$  in both patients with alpha-fetoprotein serum levels below and above 200 ng/mL. This result is in keeping with those previously obtained in similar patient populations treated with RFTA and hepatic resection, and compares favorably with the results of liver transplantation.<sup>44-46</sup> Therefore, the survival figures obtained in this and previous studies carried out in similar patient populations question the appropriateness of liver transplantation to cure solitary tumors

up to 3 cm in well-compensated cirrhosis patients, especially when the “transplant benefit” and the worldwide organ shortage are taken into consideration.<sup>46,47</sup>

This study undoubtedly has some limitations. In the current version of the ITA.LI.CA database, data regarding tumor recurrence after treatment are not available, and therefore in this study the influence of alpha-fetoprotein levels on some important composite endpoints such as recurrence plus death could not be assessed. Furthermore, as expected in our country, hepatitis virus infection was the cause of cirrhosis in most cases, and therefore it remains to be established whether these results can be generalized to HCC patients with other etiologies.<sup>48,49</sup> Lastly, although the ITA.LI.CA database includes more than 3,000 HCC patients, the selection criteria for this study were very strict, and therefore the study population was limited to 205 patients. A *post-hoc* analysis shows that this sample size had a statistical power of 22% to detect a difference between the observed 5-year survival rates of patients with alpha-fetoprotein below (61%) and above (55%) 20 ng/mL. With such survival rates, a sample size of 2,118 patients with compensated cirrhosis and single, small HCC treated with curative intent, derived from a population of more than 30,000 patients with HCC, would have been needed to achieve a power of 80%. All in all, we feel that even these figures, if framed in the context of clinical practice, confirm the bland prognostic potential of alpha-fetoprotein in the subset of patients we selected.

In conclusion, we found that serum alpha-fetoprotein has no prognostic role in compensated cirrhosis patients with a single, small HCC diagnosed during surveillance and treated with curative intent. These findings emphasize the futility of serum alpha-fetoprotein determination in a clinical setting where surveillance for HCC may provide its maximal benefit in terms of amenability to curative treatment and patients survival. New, more accurate markers are therefore needed to improve our current ability to predict the outcome of patients diagnosed with early HCC.

## Appendix

Other members of the ITA.LI.CA group: Dipartimento di Medicina Clinica, Alma Mater Studiorum, Università di Bologna, Italy: Mauro Bernardi, Maurizio Biselli, Romina Cassini, Paolo Caraceni, Marco Domenicali, Virginia Erroi, Marta Frigerio, Annagiulia Gramenzi, Barbara Lenzi. Dipartimento di Medicina Interna, dell'Invecchiamento e Malattie Nefrologiche, Azienda Ospedaliero-Universitaria di Bologna, Italy: Donatella Magalotti. Divisione di Medicina, Azienda Ospedaliera Bolognini, Seriate, Italy: Claudia Balsamo, Maria Di Marco, Elena Vavassori. Divisione di Medicina, Ospedale Treviglio-Caravaggio, Treviglio, Italy: Lodovico

Giardoni, Mario Mattiello. Dipartimento di Medicina Clinica e Sperimentale, Università di Padova, Italy: Alfredo Alberti, Angelo Gatta, Maurizio Gios. Dipartimento di Scienze Chirurgiche e Gastroenterologiche, Università di Padova, Italy: Anna Giacomini, Veronica Vanin, Caterina Pozzan, Gemma Maddalo. Dipartimento di Discipline Chirurgiche, Rianimatorie e dei Trapianti, Alma Mater Studiorum, Università di Bologna, Italy: Matteo Ravaioli, Alessandro Cucchetti. Dipartimento di Malattie Apparato Digerente e Medicina Interna, Azienda Ospedaliero-Universitaria di Bologna, Italy: Emanuela Giampalma, Rita Golfieri, Cristina Mosconi, Matteo Renzulli. Unità di Gastroenterologia, Ospedale Belcolle, Viterbo, Italy: Giorgia Ghittoni, Paola Roselli. Unità di Medicina Interna e Gastroenterologia, Università Cattolica di Roma, Roma, Italy: Giulia Bosco.

## References

- Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics 2002. *CA Cancer J Clin* 2005;55:74-108.
- Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004;127:S35-S50.
- Sherman M. Hepatocellular carcinoma: screening and staging. *Clin Liver Dis* 2011;15:323-334.
- Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004;130:417-422.
- Yuen MF, Cheng CC, Laufer IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. *HEPATOLOGY* 2000;31:330-335.
- Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* 2004;126:1005-1014.
- Santi V, Trevisani F, Gramenzi A, Grignaschi A, Mirici-Cappa F, Del Poggio P, et al. Semiannual surveillance is superior to annual surveillance for the detection of early hepatocellular carcinoma and patient survival. *J Hepatol* 2010;53:291-297.
- Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. 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.
- Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int* 2010;4:439-474.
- Bruix J, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *HEPATOLOGY* 2011;53:1020-1022.
- Tandon P, Garcia-Tsao G. Prognostic indicators in hepatocellular carcinoma: a systematic review of 72 studies. *Liver Int* 2009;29:502-510.
- Pompili M, Rapaccini GL, Covino M, Pignataro G, Caturelli E, Siena DA, et al. Prognostic factors for survival in patients with compensated cirrhosis and small hepatocellular carcinoma after percutaneous ethanol injection therapy. *Cancer* 2001;92:126-135.
- Huo TI, Huang YH, Lui WY, Wu JC, Lee PC, Chang FY, et al. Selective prognostic impact of serum alpha-fetoprotein level in patients with hepatocellular carcinoma: analysis of 543 patients in a single center. *Oncol Rep* 2004;11:543-550.
- Kim SH, Park JW, Jang JS, Kim HJ, Shin WG, Kim KH, et al. 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.
- Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999;19:329-338.
- Huo TI, Lee SD. Role of the model for end-stage liver disease and serum  $\alpha$ -fetoprotein as predictors for hepatocellular carcinoma. *Liver Int* 2006;26:1300-1301.
- Santi V, Buccione D, Di Micoli A, Fatti G, Frigerio M, Farinati F, et al. The changing scenario of hepatocellular carcinoma over the last two decades in Italy. *J Hepatol* 2012;56:397-405.
- Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646-649.
- Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982;5:649-655.
- Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer* 1954;7:462-503.
- Pirisi M, Leutner M, Pinato DJ, Avellini C, Carsana L, Toniutto P, et al. Reliability and reproducibility of the Edmondson grading of hepatocellular carcinoma using paired core biopsy and surgical resection specimens. *Arch Pathol Lab Med* 2010;134:1818-1822.
- Kim DY, Kim JW, Kuromatsu R, Ahn SH, Torimura T, Sherman M. Controversies in surveillance and early diagnosis of hepatocellular carcinoma. *Oncology* 2011;81(Suppl 1):56-60.
- Sherman M.  $\alpha$ -Fetoprotein. An obituary. *J Hepatol* 2001;34:603-605.
- Farinati F, Marino D, De Giorgio M, Baldan A, Cantarini M, Cursaro C, et al. Diagnostic and prognostic role of  $\alpha$ -fetoprotein in hepatocellular carcinoma: both or neither? *Am J Gastroenterol* 2006;101:524-532.
- Bayati N, Silverman AL, Gordon SC. Serum alpha-fetoprotein levels and liver histology in patients with chronic hepatitis C. *Am J Gastroenterol* 1998;93:2452-2456.
- Lok AS, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, et al. Des-gamma-carboxy prothrombin and alpha-fetoprotein for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010;138:493-502.
- Sterling RK, Wright EC, Morgan TR, Seeff LB, Hoefs JC, Di Bisceglie AM, et al. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. *Am J Gastroenterol* 2012;107:64-74.
- Marrero J, El-Serag HB. Alpha-fetoprotein should be included in the hepatocellular carcinoma surveillance guidelines of the American Association for the Study of Liver Diseases. *HEPATOLOGY* 2011;53:1060-1061.
- Giannini EG, Farinati F, Trevisani F. Alpha-fetoprotein in hepatocellular carcinoma surveillance: wake not the dead. *HEPATOLOGY* 2011;54:376-377.
- Nomura F, Ohnishi K, Tanabe Y. Clinical features and prognosis of hepatocellular carcinoma with reference to serum alpha-fetoprotein levels. *Cancer* 1989;64:1700-1707.
- The Cancer of the Liver Italian Program (CLIP) Investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients. *HEPATOLOGY* 1998;28:751-755.
- Rapaccini GL, Pompili M, Caturelli E, Covino M, Lippi ME, Beccaria S, et al. Hepatocellular carcinomas <2 cm in diameter complicating cirrhosis: ultrasound and clinical features in 153 consecutive patients. *Liver Int* 2004;24:124-130.
- Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. *J Hepatol* 2005;43:434-441.
- Chen TM, Huang PT, Tsai MH, Lin LF, Liu CC, Ho KS, et al. Predictors of alpha-fetoprotein elevation in patients with chronic hepatitis C, but not hepatocellular carcinoma, and its normalization after pegylated interferon alfa 2a-ribavirin combination therapy. *J Gastroenterol Hepatol* 2007;22:669-675.
- Riaz A, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, Minocha J, et al. Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. *J Clin Oncol* 2009;27:5734-5742.



36. Han K, Tzimas GN, Barkun JS, Metrakos P, Tchervenkov JL, Hilzenrat N, et al. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. *Can J Gastroenterol* 2007;21:39-45.
37. Vibert E, Azoulay D, Hoti E, Iacopinelli S, Samuel D, Salloum C, et al. Progression of alphafetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010;10:129-137.
38. Merani S, Majno P, Kneterman NM, Berney T, Morel P, Mentha G, et al. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol* 2011; 55:814-819.
39. Lencioni RA, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, et al. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. *Radiology* 2003;228:235-240.
40. Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Difference in tumor invasiveness in cirrhotic patients with hepatocellular carcinoma fulfilling the Milan criteria treated by resection and transplantation: impact on long-term survival. *Ann Surg* 2007;245:51-58.
41. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362:1907-1917.
42. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008;48:S20-S37.
43. Kojiro M, Roskams T. Early hepatocellular carcinoma and dysplastic nodules. *Semin Liver Dis* 2005;25:133-142.
44. Livraghi T, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, et al. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *HEPATOLOGY* 2008;47:82-89.
45. Santambrogio R, Opocher E, Zuin M, Selmi C, Bertolini E, Costa M, et al. Surgical resection versus laparoscopic radiofrequency ablation in patients with hepatocellular carcinoma and Child-Pugh class a liver cirrhosis. *Ann Surg Oncol* 2009;16:3289-3298.
46. Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A; OLT for HCC Consensus Group. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012;13:e11-22.
47. Vitale A, Morales RR, Zanusi G, Farinati F, Burra P, Angeli P, et al. Barcelona Clinic Liver Cancer staging and transplant survival benefit for patients with hepatocellular carcinoma: a multicentre, cohort study. *Lancet Oncol* 2011;12:654-662.
48. De Bac C, Stroffolini T, Gaeta GB, Taliani G, Giusti G. Pathogenic factors in cirrhosis with and without hepatocellular carcinoma: a multicenter Italian study. *HEPATOLOGY* 1994;20:1225-1230.
49. Stroffolini T, Andreone P, Andriulli A, Ascione A, Craxi A, Chiaramonte M, et al. Characteristics of hepatocellular carcinoma in Italy. *J Hepatol* 1998;29:944-952.

# Oncogenic Activation of Glypican-3 by c-Myc in Human Hepatocellular Carcinoma

Li Li,<sup>1,2\*</sup> Ronghua Jin,<sup>2\*</sup> Xiaoli Zhang,<sup>3</sup> Fudong Lv,<sup>2</sup> Lifeng Liu,<sup>1</sup> Daojie Liu,<sup>1</sup> Kai Liu,<sup>1</sup> Ning Li,<sup>2#</sup>  
and Dexi Chen<sup>1,2#</sup>

Glypican-3 (GPC3) is a heparan sulfate proteoglycan that has an important role in cell growth and differentiation, and its function in tumorigenesis is tissue-dependent. In hepatocellular carcinoma (HCC), the overexpression of GPC3 has been demonstrated to be a reliable diagnostic indicator. However, the mechanisms that regulate the expression and function of GPC3 remain unclear. The oncoprotein c-Myc is a transcription factor that plays a significant role in more than 50% of human tumors. We report here that GPC3 is a transcriptional target of c-Myc and that the expression of c-Myc is also regulated by GPC3, thus forming a positive feedback signaling loop. We found that the overexpression of c-Myc could induce GPC3 promoter-dependent luciferase activity in luciferase reporter experiments. Furthermore, mutational analysis identified c-Myc-binding sites within the GPC3 promoter. The exogenous overexpression of c-Myc increased the endogenous messenger RNA (mRNA) and protein levels of GPC3. Chromatin immunoprecipitation experiments revealed the binding of c-Myc to the endogenous GPC3 promoter, indicating that c-Myc can directly transcriptionally activate GPC3. Interestingly, GPC3 can also elevate c-Myc expression. Overexpression of GPC3 increased c-Myc protein levels, whereas the knockdown of GPC3 reduced c-Myc expression levels. Lastly, the elevated levels of c-Myc correlate with the overexpression of GPC3 in human HCC samples. **Conclusion:** These data provide new mechanistic insight into the roles of GPC3 and of c-Myc in the development of HCC. (HEPATOLOGY 2012;56:1380-1390)



See Editorial on page 1194

Primary hepatocellular carcinoma (HCC) is the world's fifth most common cancer, and the third most common cause of cancer deaths.<sup>1</sup> HCC is a common malignant tumor, and causes over 100,000 HCC deaths per year in China.<sup>2</sup> Only 10%-20% of total HCCs can be treated in the early stage by surgery. Most HCCs are diagnosed at late stages, and as a result they progress rapidly, are difficult to treat, and exhibit poor prognosis. Early diagnosis and treatment are critical factors for improving the survival of HCC patients.

Recent research has shown that glypican-3 (GPC3) is a specific and sensitive biomarker for the diagnosis of HCC.<sup>3</sup> GPC3 is a proteoglycan that is localized on the cell surface. The abnormal expression of GPC3 in HCC was first reported by Hsu et al.<sup>4</sup> in 1997. In that study, 75% of HCC patients were found to overexpress GPC3 messenger RNA (mRNA), whereas patients with benign liver disease or normal livers exhibited no expression of GPC3 mRNA or protein. Soluble GPC3 protein can be detected in the serum of 50% of HCC patients, demonstrating its value as a diagnostic marker for HCC.<sup>5</sup> The expression of GPC3, its receptor, and of other growth factors coordinate signal transduction pathways that regulate cellular morphology and a variety of cellular behaviors, such as adhesion, proliferation, migration, survival,

Abbreviations: GPC3, glypican-3; HCC, hepatocellular carcinoma; MD, moderately differentiated; PD, poorly differentiated; WD, well differentiated.

From the <sup>1</sup>Beijing Institute of Liver Disease, Beijing, China; <sup>2</sup>Department of Infectious Medicine, Beijing You'an Hospital, Capital Medical University, Beijing, China; and <sup>3</sup>Department of Molecular and Medical Genetics, Oregon Health & Sciences University, Portland, OR.

Received December 21, 2011; accepted May 22, 2012.

Supported by National Natural Science Foundation of China (30910103915, 81071843), Beijing Natural Science Foundation (7101005), and Twelve-fifth Key Science and Technology Five Year Plan of China (2012ZX10001-003, 2012ZX10001-004).

\*These two authors contributed equally to this work.

#These two authors share senior authorship.