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The Predictive Power of Serum α-Fetoprotein and Des-γ-Carboxy Prothrombin for Survival Varies by Tumor Size in Hepatocellular Carcinoma

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Alpha-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) are frequently used as tumor markers in hepatocellular carcinoma (HCC). The authors hypothesized different patient populations with varying tumor sizes would influence the predictive power of tumor markers for survival in HCC patients. The authors investigated the influence of tumor size on predictive powers of AFP and DCP.

181 patients underwent hepatectomy for HCC from 2003 to 2008 at Kobe University Hospital. Tumor markers were measured before and at 1 month post-hepatectomy.

The Cox proportional-hazards model revealed that preoperative serum AFP was associated with survival; its effects depended on tumor size. Hazard ratios (HRs) for preoperative AFP were maximum for medium-sized HCC, and for DCP, HRs were maximum in small-sized tumors. Post-hepatectomy, both tumor markers were associated with survival, revealing significant interactions with tumor size. HRs for postoperative AFP were greater than 1 for relatively wide range tumors (3–11 cm). HRs for postoperative DCP increased with tumor size, with a strong prognostic predictive power for tumors >5 cm.

The predictive power of serum tumor markers varied by tumor size in HCC patients. By selecting the appropriate tumor marker, its predictive power can be improved.

INTRODUCTION

Hepatocellular carcinoma (HCC) continues to be a leading cause of cancer-related deaths worldwide, with the highest incidence in Asian and developing countries (13). There is, however, a marked geographic variation in the incidence of HCC, ranging from 2.8 new cases per 100,000 persons per year in the United States to more than 30 new cases per 100,000 persons per year in Hong Kong (23). Although the incidence of HCC is relatively low in the United States, it has increased throughout the last decade due to the hepatitis C virus and non-alcoholic steatohepatitis (2).

Despite recent advances in the treatment of HCC, there are limited possibilities for a cure. Hepatectomy or liver transplantation is most often used as a curative strategy, but only a small proportion of HCC patients can receive these treatments due to multifocal tumors or underlying poor hepatic function. In addition, more than 70% of HCC patients experience recurrence within 5 years despite curative treatment. Therefore, many studies have been conducted to identify diagnostic markers to detect early-stage HCC and to identify prognostic predictors for HCC. Serum α-fetoprotein (AFP) became the first tumor marker to be used diagnostically despite its limited specificity (27). Serum des-γ-carboxy prothrombin (DCP), also known as protein induced by vitamin K absence or antagonist (PIVKA-II), has been regarded as an alternative tumor marker for the diagnosis of HCC (17, 22) and a prognostic marker associated with portal vein invasion, tumor size, intrahepatic metastasis, and recurrence and survival after treatment in HCC (11, 12, 16). Thus, serum AFP and DCP are regarded as useful diagnostic and prognostic markers in HCC. However, these findings cannot be naively generalized because many contradictory results exist regarding the clinical implications of serum AFP and DCP, particularly between Eastern and Western countries.

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In general, serum tumor markers play different clinical roles in the treatment of HCC. First, they are measured in patients considered to be at risk of developing HCC for screening and diagnosis. The American Association for the Study of Liver Diseases guidelines recommend measuring serum AFP levels and performing ultrasonography for surveillance (3). Second, these markers are used as tools for monitoring the effect of treatment and the progression of HCC. Serum AFP and DCP levels are usually normalized after the treatment of HCC with curative intent, and re-elevation of these markers implicates disease recurrence. Third, they are used as prognostic predictive tools in patients with HCC. For this purpose, the Cancer of the Liver Italian Program (CLIP) staging system assigns a score to serum AFP (28).

Controversy still exists, however, regarding the clinical implications of these tumor markers, especially regarding their use as predictors of survival, with positive and negative results for both AFP and DCP. Most positive results for the predictive power of serum AFP have been published from Western countries (6, 9), while most positive results for serum DCP come from Eastern countries (5, 14, 24, 25). Although, several factors including etiology and geography might be implicated as a cause of this controversy, we focused on the tumor size in this study. In fact, there are significant differences in patient population and tumor size of HCC at diagnosis from country to country (6, 23); in this study, we focused on tumor size to resolve the discrepancy between the predictive powers of AFP and DCP. We assumed that each tumor marker has a different predictive power because each tumor marker has different production and release mechanisms (5, 9, 24).

We hypothesized that tumor size would influence the predictive power of serum tumor markers for survival in patients with HCC. To the best of our knowledge, no reports have thus far evaluated the association between tumor size and the predictive power of serum tumor markers for survival in the treatment of HCC. Therefore, in this study, we aimed to determine the influence of tumor size on the predictive power of AFP and DCP for survival and identify the most appropriate prognostic predictors for HCC treatment.

METHODS

Patients

A prospectively maintained clinical database of patients undergoing hepatectomy for HCC was used for this study. In a 6-year period from January 2003 to December 2008, 181 consecutive patients had undergone an initial hepatectomy for HCC at Kobe University Hospital, Kobe, Japan. Extrahepatic tumor metastasis was excluded prior to surgery in all patients.

Tumor Markers

Serum AFP and DCP levels were measured at the time of preoperative examination within 7 days prior to hepatectomy and at 1 month after hepatectomy as postoperative serum tumor markers. Serum AFP levels were measured by the commercially available chemiluminescence immunosorbent assay kit Lumipulse® G AFP-N (Fujirebio Inc., Tokyo, Japan). Serum DCP levels were measured by the commercially available chemiluminescence immunosorbent assay kit Lumipulse® PIVKA-II Eisai (Eisai Co., Ltd., Tokyo, Japan).

Treatment and Surveillance

To evaluate liver function, all patients underwent preoperative liver chemistry tests, Child-Pugh grading, and measurements of 15-min retention rate for indocyanine green (ICGR15). The surgical procedure was selected according to the tumor location and liver function. The assessment of liver function was primarily based on the comprehensive judgment of the ICGR15 and general serum liver chemistry results, and the final decision regarding the extent of resection was taken after evaluating the fibrosis score of the liver in laparotomy. Resection of two or more liver segments was defined as major hepatectomy. Curative hepatectomy was defined as complete removal of the tumor with negative microscopic margins and no residual tumors detected on postoperative imaging studies on ultrasonography or contrast-enhanced computed tomographic (CE-CT) studies within 1 month postoperatively. In addition, patients who had a tumor thrombus either in the portal trunk or in the major hepatic veins were excluded from curative hepatectomy, considering the high risk of macroscopic and microscopic residual liver tumors. Postoperative mortality was defined as death in the hospital after hepatectomy during the first admission. Any complications requiring medication or an interventional procedure was considered postoperative morbidity. All patients were followed at least every 3 months in the outpatient clinic with serum liver chemistry tests and by monitoring their serum AFP and DCP levels. CE-CT or magnetic resonance imaging (MRI) was performed at 1 month and 3 months postoperatively, and at least every 6 months thereafter.

Statistical analysis

Baseline patient characteristics were summarized using medians and quartiles and compared across patient groups defined by survival outcomes using Wilcoxon's rank-sum tests (continuous) and Fisher's exact tests (discrete). Survival functions were estimated by the Kaplan-Meier method for graphical presentation. We fitted multivariable Cox proportional-hazards regression models to investigate the usefulness of the preoperative and postoperative levels of serum AFP and DCP. Tumor size was included in the model with a regression spline to allow for possible non-linear association with the survival outcome. Interaction with the primary markers was included to allow for serum AFP and DCP to have different magnitudes of association with the survival outcome depending on the tumor size. The marker values were logarithmically transformed prior to fitting the model to reduce the skewness of the distributions. Their association with survival was tested using the Wald test. No other variables were included in the models as the sample sizes were small, making stable estimation of the regression coefficients impossible. For each model (preoperative and postoperative), we checked the proportional hazard assumptions for all the variables in each model using scaled Schoenfeld residuals and found no violations. We estimated adjusted hazard ratios as a measure of the treatment effect computed at the upper quartile, with the lower quartile as the baseline for each tumor marker. All significance tests were 2-sided, and a p-value of 0.05 was considered statistically significant. All statistical analyses were conducted with the statistical program R (version 2.15).

RESULTS

Patient Characteristics and Tumor Markers

Table I shows the patient and tumor characteristics at the time of hepatectomy. Among the 181 patients, 155 (86%) were men, the median age of the patients was 66.7 (quartiles 59.3, 72.7), and the mean size of the tumor was 6.5 cm (standard deviation (SD) = 4.3). The median follow-up was 28.0 months, during which time 76 (42%) patients died. Univariable analyses showed that serum AFP (preoperative and postoperative) and DCP (preoperative and postoperative) levels were significantly associated with patient survival. Additionally, number of tumors (solitary/multiple), tumor size, vascular invasion (macro/micro/none), and curability of surgery (yes/no) were also associated with survival (Table II).

Table I. Patient characteristics

Variable		Alive N = 105	Dead N = 76	p-value
Age		66.7* (8.8)	62.4* (12.9)	0.01*
	60 and younger	25% (26)	37% (28)	
	61-70	39% (41)	37% (28)	
	71 and older	26% (38)	26% (20)	
Sex	Female	16% (17)	12% (9)	0.52
HBsAg	Positive	28% (29)	33% (25/75)	0.42
HCV-Ab	Positive	22% (20/93)	27% (17/64)	0.57
Cirrhosis		58% (61)	63% (48)	0.54
Child-Pugh	Grade A	97% (102)	93% (71)	0.28
	Grade B	3% (3)	7% (5)	
	Grade C	0% (0)	0% (0)	
Liver Surgery	Major Hepatectomy	25% (26)	46% (35)	0.004
Number of Tumors	Multiple	29% (30)	68% (52)	< 0.001
Tumor Size (cm)		5.0* (2.9)	8.6* (5.0)	< 0.001*
Vascular Invasion	Macro	5% (5)	28% (21)	< 0.001
	Micro	30% (31)	47% (36)	
	None	65% (69)	25% (19)	
Curability of Surgery	Curative	91% (96)	50% (38)	< 0.001
Pathology	Well Differentiated	14% (15)	6% (5)	0.08
	Moderately Differentiated	78% (82)	78% (59)	
	Poorly Differentiated	8% (8)	16% (12)	
Pre-Ope AFP (ng/ml)		17 (5, 224)	114 (15, 3177)	< 0.001
	≥20	48% (49)	52% (54)	
Pre-Ope DCP (mAU/ml)		339 (40, 2526)	1547 (265, 18156)	< 0.001
	≥40	44% (40)	56% (51)	
Post-Ope AFP (ng/ml)		5 (3, 9)	15 (5, 146)	< 0.001
	≥20	27% (12)	73% (33)	
Post-OpeDCP (mAU/ml)		18 (14, 22)	34 (20, 191)	< 0.001
	≥40	24% (11)	76% (34)	

Categorical variables are shown with % (N), and continuous variables are shown with median (quartiles) or mean (sd) where indicated by an asterisk

Univariate tests were conducted with Fisher's exact test (categorical) and Wilcoxon rank sum test (continuous variables) or t-test when indicated by an asterisk

Table II. Factors associated with survival

Variable		Number of patients	3 year OS	p-value
Sex	Male	155	60.5	0.369
	Female	26	65.1	
Pre-Ope AFP (ng/ml)	< 1000	140	68.9	<0.001
	≥ 1000	41	35.3	
Pre-Ope DCP (mAU/ml)	< 100	49	89.2	<0.001
	≥ 100	132	50.2	
Number of Tumors	Solitary	99	77	<0.001
	Multiple	82	41.6	
Tumor size (cm)	< 5	80	84.8	<0.001
	≥ 5	101	43.5	
Pathology	Well and Moderate HCC	161	64.1	0.035
	Poorly HCC	20	39.4	
Notumorous liver	cirrhosis	109	62.9	0.856
	chronic hepatitis or normal	72	58.4	
Macroscopic vascular invasion	positive	26	2	<0.001
	negative	155	68.4	
Microscopic vascular invasion	positive	95	40.5	<0.001
	negative	86	84.3	
Type of hepatectomy	curative	134	75.3	<0.001
	reductive	47	20.9	
Post-Ope AFP (ng/ml)	< 1000	168	65.3	<0.001
	≥ 1000	12	16.7	
Post-Ope DCP (mAU/ml)	< 100	148	70.2	<0.001
	≥ 100	32	18.8	

Preoperative model

The Cox proportional-hazards model revealed that preoperative levels of serum AFP were associated with survival ($p = 0.029$). As expected, tumor size by itself was highly associated with the outcome ($p < 0.001$). The interaction between tumor size and preoperative serum AFP was also significant ($p = 0.031$), and the effects of preoperative serum AFP appeared to depend on the size of the tumor, being maximum for medium-sized tumors. The Cox proportional-hazards model revealed that preoperative serum DCP levels were not associated with survival, and the interaction between tumor size and preoperative DCP levels was also not significant.

Figure 1 shows the hazard ratios comparing high AFP (650) to low AFP (10) levels and comparing high DCP (4,000) to low DCP (60) levels at specific tumor sizes ranging from 1.5 to 15.0 cm. These values for AFP and DCP are approximately at the upper and lower quartiles. Confidence intervals of the hazard ratio estimates are wide where the sample data are scarce (tumor size < 5 cm and tumor size > 13 cm). Hazard ratios of preoperative serum AFP levels were maximum in patients with medium-sized HCC measuring between 5 and 8 cm. Hazard ratios of AFP in this tumor size were greater than twice the ratios in large- and small-sized tumors. Hazard ratios of DCP were maximum in small-sized tumors measuring between 1 cm and 3 cm, being 3 times higher than those in medium- and large-sized tumors.

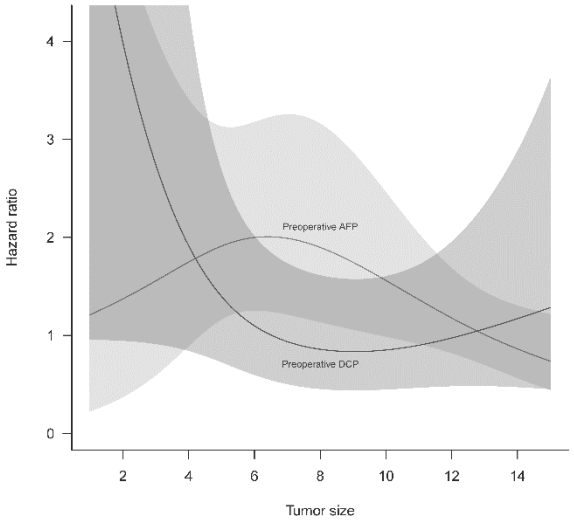

Fig.1. Multivariable Cox proportional-hazards regression model of the preoperative levels of serum AFP and DCP

Postoperative model

A model with the same structure was fitted for postoperative serum AFP and DCP levels. Both serum AFP and DCP were highly associated with survival ($p < 0.001$ for both markers). Their interactions with tumor size were also significant ($p < 0.001$ for AFP and $p = 0.049$ for DCP). Non-linear association between tumor size and survival was also noted to be significant, indicating complex quantitative relationships between the markers and survival for different tumor sizes. Figure 2 shows the hazard ratios comparing the upper and lower quartiles of AFP (20 vs. 4) and DCP (40 vs. 15) at specific tumor sizes ranging from 1.5 to 15.0 cm. The hazard ratios associated with AFP were significantly greater than 1 for relatively wide range tumors (between 3 and 11 cm); however, this effect diminished for tumors measuring >12 cm. Hazard ratios for postoperative serum DCP levels increased with tumor size, and serum DCP levels was a strong prognostic predictor for patients with tumor measuring >5 cm.

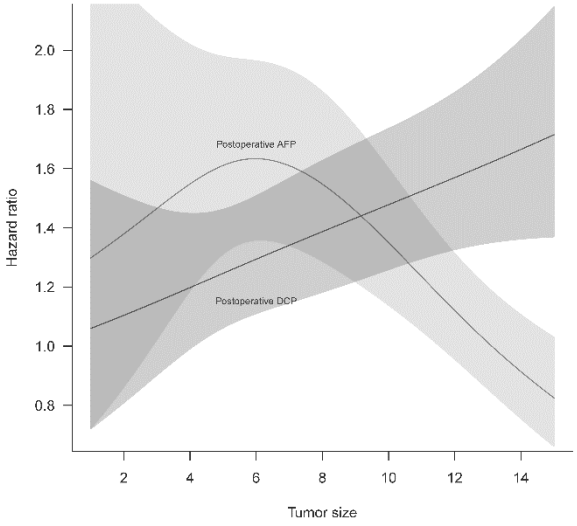

Fig.2. Multivariable Cox proportional-hazards regression model of the postoperative levels of serum AFP and DCP

DISCUSSION

In this study, we demonstrated that the predictive powers of serum AFP and DCP for survival before and after hepatectomy differed from each other and varied based on the tumor size in patients with HCC. We believe that our findings can resolve the existing discrepancy among reports regarding the predictive power of tumor markers for survival, providing valuable information about an appropriate prognostic predictor and adequate follow-up methods for patients with HCC.

Apart from roles in screening and diagnosis, serum tumor markers are used as prognostic predictors in the treatment of HCC (11, 12, 14, 15, 16, 25, 30). For this purpose, serum AFP levels are measured and used worldwide and integrated into the CLIP score, i.e. In contrast, serum DCP has not achieved a similar worldwide acceptance as a useful predictor. In Japan, serum DCP levels have been routinely measured since the 1990s (10) and have been used as a prognostic indicator for patients with HCC based on studies of Japanese patient populations; however, in Western countries, DCP has not been established as a tumor marker for HCC nor is it recommended as a prognostic indicator of HCC. To the best of our knowledge, this discrepancy has not been fully elucidated. Therefore, in this study, we evaluated the influence of tumor size on the predictive power of serum AFP and DCP for survival.

Interestingly, the reported tumor size at diagnosis differs considerably between Western and Eastern countries (6, 23). According to Esnaola et al. (6), the median tumor size for patients treated in the United States was 8 cm, as compared to 6 cm for patients treated in France and only 3.5 cm for patients treated in Japan. Thus, studies conducted in Japan tend to have a larger percentage of patients with small-sized HCC than studies conducted in Western countries. In fact, Japanese studies that demonstrated the positive predictive power of serum DCP levels reported the mean diameters of HCCs as <3 cm and 4 cm (4, 19). On the other hand, a US study included HCC tumors measuring >5 cm in diameter in more than half of its patients and failed to show an association between serum DCP and patient survival (4). In addition, this study also failed to show an association between serum DCP levels and patient survival in the whole study population, possibly because this study contained a large number of patients with advanced HCC and a mean tumor diameter of 6.5 cm. These data support our findings that in small-sized tumors, DCP appears to have good predictive power for survival (Fig. 1).

We believe that our findings will aid in clarifying the discrepancies in the utility of serum DCP as a prognostic marker for HCC.

Thus far, we have no definitive answer as to why preoperative serum DCP levels tend to have positive predictive power only in patients with small-sized HCC. Many studies concerning the relationship between serum DCP levels and various clinicopathological features of HCC have suggested that elevated serum DCP is associated with worse tumor behavior, including vascular invasion, HCC recurrence, intrahepatic metastasis and poor differentiation of HCC cells (8, 11, 16, 19, 21, 25). In addition, recent molecular biological studies have demonstrated serum DCP induces the proliferation of HCC cells (26) as well as angiogenesis resulting in cancer progression (7, 29). Unfortunately, none of these findings directly answer the question regarding DCP levels and tumor size. One possible explanation may be that tumors that show elevated serum DCP levels despite a small tumor size have higher malignant potential—such as vascular invasion, poor differentiation, etc.—than medium- and large-sized tumors with similar DCP levels. However, this remains in the realm of speculation, and further studies are required to answer this question.

Contradictory findings also exist regarding the predictive power of serum AFP levels measured before treatment. Carr et al. reported a significant association between serum AFP levels before treatment and patient survival (4), while Kim et al. reported that serum AFP level was not a valuable independent prognostic factor for HCC patients with a mean tumor size between 4 and 5 cm before treatment (15). Nagaoka et al. also reported that serum AFP level was not a useful prognostic indicator for HCC patients with a mean tumor size <3 cm (19). However, the present study could demonstrate a statistical relationship between preoperative serum AFP level and patient survival in the entire study population. It is notable that the mean tumor diameter in our study was 6.5 cm, being greater than that in the previously mentioned reports. These data supported our hypothesis that the predictive power of preoperative serum AFP level is associated with tumor size, i.e., preoperative serum AFP level has a good predictive power only for patients with medium-sized tumors (5–9 cm). Multiple reasons may be responsible for this observation. One possible explanation is that AFP tends to lose predictive power in patients with small-sized HCC because serum AFP levels can also increase to a certain degree in cases of benign liver disease such as chronic hepatitis and cirrhosis.

After treatment for HCC, elevated serum AFP and DCP levels are usually normalized; however, they often increase again with recurrence of HCC. Therefore, serum AFP and DCP levels are used to detect tumor recurrence and progression and are used as prognostic indicators in HCC patients following treatment. Post-operative high tumor marker level might reflect aggressive characters of remnant tumors undetected by intraoperative findings or preoperative radiological studies or after non-curative surgery. Post-operative tumor markers are more important than preoperative tumor markers in that the aggressive character and the effectiveness of surgery can be perceived. However, the clinical significance of post-treatment serum AFP and DCP levels in predicting patient survival remains contradictory. Nagaoka et al. reported that postoperative serum AFP levels are more useful in predicting recurrence following hepatectomy than postoperative serum DCP levels (19). Nanashima et al. reported that postoperative serum DCP levels were associated with prolonged survival, while serum AFP levels were not correlated with patient prognosis (21). This discrepancy might be explained by our findings. Postoperative serum AFP level is a good prognostic indicator in patients with small-sized and relatively wide range HCC (between 3 and 11 cm), whereas serum DCP is a good prognostic indicator only for patients with large-sized HCC (>5 cm) (Fig. 2).

Because of the difference in the treatment results and roles of the serum tumor markers, several authors have suggested that HCC may represent different forms of the disease in different regions of the world (1, 6, 18). However, our findings suggest that these observed disparities may be explained by the differences in tumor size at the time of diagnosis. By selecting the appropriate serum tumor marker according to the tumor size, we expect to observe an improvement in the prognostic power of the serum tumor marker for HCC treatment. Based on our results, preoperative serum AFP levels in patients with medium-sized tumors and serum DCP levels in patients with small-sized tumors are recommended as survival predictors (Fig. 1). On the other hand, for postoperative prognoses, postoperative serum AFP levels in patients with small- and medium-sized tumors and postoperative serum DCP in patients with large-sized tumors should be selected as good survival predictors (Fig. 2).

The number of tumors is associated with preoperative AFP, DCP, and tumor size indicated by Spearman's correlations of 0.19, 0.30, and 0.26, respectively (data not shown). However, patients' survival does not seem to be directly influenced by the number of tumors (log rank test $p = 0.073$). In a bigger study, we would attempt to adjust for the influence of tumor count in the model.

Our current study has certain limitations. First, the sample size was relatively small. Second, all of our patients underwent hepatectomy; therefore, we lacked the comparative data for patients who were not treated at all or were treated by other modalities. Third, all of our subjects were Japanese. The observations presented here need to be verified in a multicenter, multinational study.

CONCLUSION

Our findings suggested that the predictive power of serum AFP and DCP levels for survival varied according to tumor size in HCC patients. By selecting the appropriate tumor markers based on tumor size, their predictive power for survival can be improved.

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