RESEARCH ARTICLE

Elevated PIVKA-II is Associated with Early Recurrence and Poor Prognosis in BCLC 0-A Hepatocellular Carcinomas

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Abstract

Background: To investigate the prognostic value of serum PIVKA-II (prothrombin induced by the absence of vitamin K or antagonist-II) in BCLC (Barcelona Clinic Liver Cancer) 0-A hepatocellular carcinoma (HCC) patients after curative resection. Materials and Methods: Preoperative sera were collected from 140 patients with BCLC 0-A HCCs undergoing curative resection during 2011-2012 in Zhongshan Hospital. Follow-up ended on November 2013. ELISA was used to detect the serum concentrations of preoperative PIVKA-II. The prognostic value of PIVKA-II and other clinicopathological factors was analyzed by the Kaplan-Meier method and the multivariate Cox proportional hazards model. Results: During follow-up, 39 of 140 patients suffered recurrence and the 1-year recurrence rate was 27.9%. The high-PIVKA-II expression group had lower 1-year time to progression (TTP) compared with the low-expression group (54.8% *vs* 20.2%, $p<0.001$). Patients with high preoperative PIVKA-II expression showed a relatively higher risk of developing postoperative recurrence than those with low expression in the low-recurrence-risk subgroups, including α-fetoprotein ≤400ng/mL (45.4% *vs* 16.7%; $p=0.006$), tumor size ≤5 cm (54.2% *vs* 18.1%; $p<0.001$), single tumor (56.0% *vs* 19.1%; $p<0.001$), absence of satellite lesions (53.3% *vs* 19.8%; $p=0.001$), absence of vascular invasion (52.6% *vs* 14.9%; $p=0.002$), and Edmondson stage I/II (60.9% *vs* 20.3%; $p<0.001$). PIVKA-II was the strongest independent prognostic factor for TTP (hazard ratio, 2.877; 95% CI 1.524-5.429; $p=0.001$). Conclusions: Elevated PIVKA-II is associated with early recurrence of BCLC 0-A HCC after curative resection and can be considered a novel prognostic predictor.

Keywords: Hepatocellular carcinoma - PIVKA-II - BCLC; prognosis - recurrence

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, with an increasing incidence in recent years and dismal outcomes (Siegel et al., 2012). Surgery remains the most effective treatment with curative potential, but only 10-20% of HCC patients are eligible for surgical intervention owing to the lack of effective early diagnosis. For the remaining patients, transcatheter arterial chemoembolization (TACE), radiotherapy (RT), or sorafenib are common treatment choices (Bruix et al., 2011). Despite improvements in surveillance and clinical treatment strategies, the prognosis of HCC remains poor because of the high incidence of recurrence and metastasis. Traditional clinicopathological parameters such as tumor morphology, histopathological features, radiological modalities, and tumor staging system offer limited information for predicting postoperative recurrence (Cha et al., 2003). Therefore, it is imperative to develop new approaches for discriminating high-risk factors in patients with recurrence.

The Barcelona Clinic Liver Cancer (BCLC) staging system has become widely accepted in clinical practice and is recommended for prognostic prediction and treatment allocation. It is also used in many clinical trials of new drugs for HCC. The BCLC staging system can be applied to most HCC patients, as long as consideration is given to special subpopulations (e.g., liver transplantation). Patients at very early Stage 0 and early Stage A are optimal candidates for a radical approach. Radical therapy can change the course of HCC, leading to favorable long-term outcomes (Bruix et al., 2011). Patients with BCLC 0-A cancer are generally thought to have good prognosis after surgical resection, but many studies have reported variable results (Takayama et al., 2008; Santi et al., 2010). Potentially important prognostic factors such as tumor size, multifocal tumors, and vascular invasion may play a major role in these patients.

Tumor markers are used in the diagnosis and staging of cancer, in monitoring therapeutic effectiveness, in detecting recurrence, and in predicting prognosis. α-Fetoprotein (AFP) was first introduced as a serological marker for HCC and has since been used in clinical practice both for diagnosis and as an indicator of tumor response to treatment (Gupta et al., 2013; Shi et al., 2014; Wang et al., 2014). Previous studies have demonstrated that PIVKA-II (prothrombin induced by the absence of vitamin K or antagonist-II) is also an effective marker

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for HCC, and that an elevated PIVKA-II level appears to predict worse tumor behavior.

Additionally, recent molecular studies of PIVKA-II have revealed the usefulness of this molecule as a diagnostic marker, as well as its significant role in cancer progression (Wang et al., 2009a; Bertino et al., 2012; Matsubara et al., 2012). However, it remains to be confirmed whether the prognostic predictive value of the PIVKA-II level can be generalized to patients with BCLC 0-A HCC undergoing surgery.

The purpose of the present study was to explore the prognostic significance of PIVKA-II level in patients with BCLC 0-A HCC undergoing surgery, and to analyze the value of PIVKA-II level for predicting the risk of early recurrence after hepatectomy.

Materials and Methods

Study design

From March 2011 to October 2012, 261 HCC patients were recruited into this prospective study and underwent curative resection. Curative resection was defined as removal of all recognizable tumors. HCC was diagnosed on the basis of tumor markers and a combination of typical imaging findings on ultrasonography, and dynamic contrast-enhanced computed tomography (CT), according to the American Association for the Study of Liver Diseases (AASLD) guidelines. Disease was stratified according to the 2011 BCLC staging classification. We only focused on patients with BCLC 0-A HCC. Patients with pathologically proven BCLC B-D cancer, or patients who were lost to follow-up after hepatectomy were excluded. The demographic, preoperative laboratory, and pathology data of all patients were collected from electronic medical records. Liver function was evaluated by the Child-Pugh classification system. Of the 261 patients, 140 were finally entered into the analyses, and 121 were excluded for the following reasons: 75 patients were classified as BCLC B-D, and 46 were lost to follow-up after discharge.

For evaluation of prognostic value, follow-up was completed in November 2013. Time to recurrence (TTR) was defined as the interval between surgery and the diagnosis of any type of recurrence, with intrahepatic recurrence and extrahepatic metastasis defined as the end points for TTR. Progression-free survival (PFS) was defined as the time between the date of receiving treatment and the date of clinical disease progression, or the date of the last follow-up visit if progression did not occur during follow-up. Approval for the use of human subjects was obtained from the Research Ethics Committee of Zhongshan Hospital, and informed consent was obtained from each individual enrolled in this study.

PIVKA-II determination

The serum concentrations of PIVKA-II were determined by enzyme immunoassay (Eisai Co., Tokyo, Japan; cutoff value 40 mAU/mL) according to the manufacturer's instructions. The level of PIVKA-II was measured when patients were initially diagnosed with HCC, and regular follow-up measurements after treatment were done in some patients.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 (IBM, Chicago, IL, USA). Experimental values are presented as mean±SEM. χ^2 tests and Fisher's exact probability test were used for comparison between groups, as appropriate. The relationship between TTR or PFS and the level of PIVKA-II was analyzed by Kaplan-Meier survival curves and the log-rank test. Results are expressed as hazard ratios (HRs) from the Cox models, along with 95% CI. Receiver operating characteristic (ROC) curve analysis was used to determine the predictive value of the parameters, and the differences in the area under the curve (AUC) were detected. Two-sided p values <0.05 were considered statistically significant.

Results

Patient demographics and tumor characteristics

Patient demographics are listed in (Table 1). The main cause of HCC was chronic infection with hepatitis B virus (n=127, 90.7%). Most patients were Child-Pugh class A and only seven patients were class B. Fourteen patients were classified as BCLC 0, and the remaining 126 were BCLC A. By the time of analysis, early recurrence had occurred in 39 of 140 patients, with a mean follow-up time

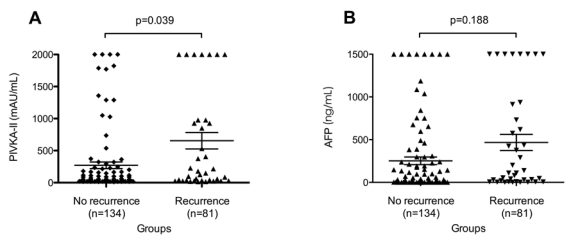

Figure 1. PIVKA-II Levels Correlated Significantly with Early Recurrence. A) Distribution of PIVKA-II levels in early recurrent patients (◆), nonrecurrent patients (▲) (p=0.039). B) Distribution of AFP levels in early recurrent patients (▲), nonrecurrent patients (▼) (p=0.188)

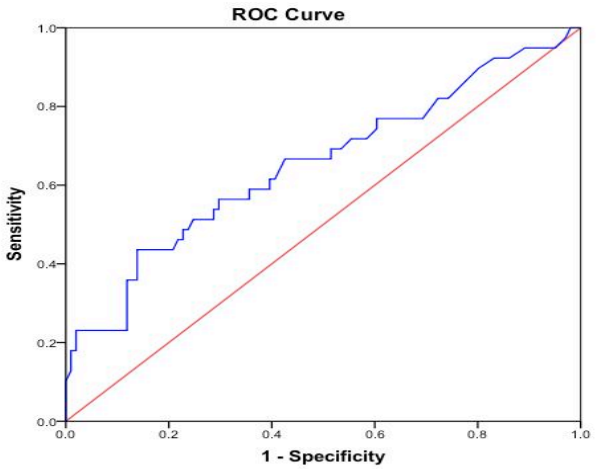

Figure 2. ROC Curves of PIVKA-II for Diagnosis of HCC Early Recurrence. AUC of PIVKA-II was 0.653

Table 1. Clinical Characteristics of BCLC Stage 0-A HCC Patients and Correlation with PIVKA-II Levels

Variable	No. of patients -140	PIVKA-II <373.5 mAU/mL (n=109)	PIVKA-II ≥373.5 mAU/mL (n=31)	*p*
Age, years				0.624
≤50	49	37	12	
>50	91	72	19	
Sex				0.285†
Male	116	88	28	
Female	24	21	3	
HBsAg				0.766†
Negative	13	10	3	
Positive	127	99	28	
Child-Pugh score				0.607†
A	133	103	30	
B	7	6	1	
Liver cirrhosis				0.130†
No	28	25	3	
Yes	112	84	28	
ALT, U/L				0.223
≤75	94	76	18	
>75	46	33	13	
AFP, ng/mL				0.485
≤400	106	84	22	
>400	34	25	9	
No. of tumors				0.234
Single	118	94	24	
Multiple	22	15	7	
Tumor size, cm				0.442
≤5	119	94	25	
>5	21	15	6	
Tumor encapsulation				0.71
Complete	49	39	10	
None	91	70	21	
Satellite lesion				0.684
No	131	101	30	
Yes	9	8	1	
Vascular invasion				0.492
No	93	74	19	
Yes	47	35	12	
Edmondson stage				0.85
I-II	102	79	23	
III-IV	38	30	8	
Recurrence				<0.001
No	101	87	14	
Yes	39	22	17	

*ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; †Fisher's exact test

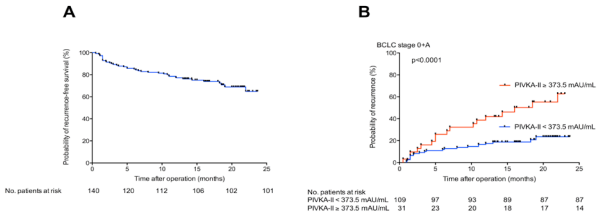

Figure 3. PIVKA-II levels Correlated Significantly with Early Recurrence in BCLC Stage 0-A Patients. A) Overall recurrence-free survival curve of 140 BCLC stage 0-A patients who underwent curative hepatic resection B) Kaplan-Meier analysis for time to recurrence in HCC patients with PIVKA-II ≥373.5mAU/mL or <373.5m AU/mL, preoperatively

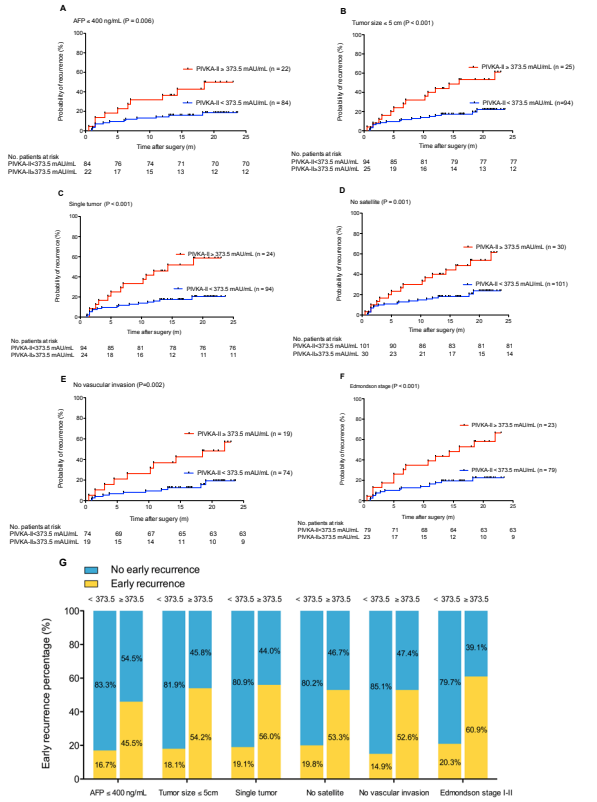

Figure 4. Kaplan-Meier Analysis of PIVKA-II Levels in Subgroups of BCLC 0-A HCC Patients. The prognostic value of PIVKA-II levels was significant in A) AFP ≤400ng/mL and B-F) clinical low-recurrence-risk subgroups, including B) tumor size ≤5cm, C) single tumor, D) no satellite lesions, E) no vascular invasion, and F) Edmondson stage I/II. G) Recurrent rates of patients with PIVKA-II levels <373.5mAU/mL versus ≥373.5mAU/mL in the subgroups for AFP ≤400ng/mL, tumor size ≤5cm, single tumor, no satellite lesion, no vascular invasion, and Edmondson stage I/II

of 18.78±0.69 months (95% CI 17.42-20.14 months). The levels of PIVKA-II were significantly higher in patients with early recurrence than in those without early recurrence (median 28.25 mAU/mL, interquartile range 55-314.25; mean 819.8, SD 2959.69 mAU/mL; p=0.039; (Figure 1). As expected, the median concentration of AFP in serum did not differ between patients with and without early recurrence (p=0.188). (Figure 2) shows the ROC curves for all patients with and without early recurrence. The area under the ROC curves (AUROC) for PIVKA-II was 0.653. The cutoff value was 373.5 mAU/mL. One hundred and nine patients had PIVKA-II level <373.5 mAU/mL, and 31 had ≥373.5 mAU/mL. Levels of PIVKA-II were significantly higher in patients with recurrence than in those without recurrence. Recurrence was observed in 17 of 31 patients with preoperative PIVKA-II level ≥373.5 mAU/mL, whereas only 22 of 109 patients with <373.5 mAU/mL had recurrence (p<0.001, Table 2).

Survival

The 1- and 2-year disease-free survival rates were 77.1 and 72.1%, respectively (Figure 3A). Patients with PIVKA-II level >373.5 mAU/mL had significantly shorter TTR and higher recurrence rates (54.8% *vs* 20.2%) than those with PIVKA-II level <373.5 mAU/mL (p<0.0001) (Figure 3B). Independent predictors of tumor recurrence by univariate analysis were associated with PIVKA-II

Table 2. Univariate and Multivariate Cox Proportional Regression Analysis of Factors Associated with Recurrence

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age, >50 *vs* ≤50 years	0.538 (0.286-1.013)	0.055	NA	NA
Sex, male *vs* female	1.323 (0.517-3.384)	0.559	NA	NA
HBsAg, positive *vs* negative	1.388 (0.332-5.799)	0.653	NA	NA
Liver cirrhosis, yes *vs* no	3.271 (1.007-10.627)	0.049	NA	NA
Child-Pugh score, B *vs* A	2.007 (0.616-6.543)	0.248	NA	NA
ALT, >75 U/L *vs* ≤75 U/L	1.272 (0.667-2.427)	0.465	NA	NA
AFP, >400ng/mL *vs* ≤400ng/mL	2.123 (1.112-4.053)	0.023	NA	NA
No. of tumors, multiple *vs* single	1.507 (0.711-3.192)	0.285	NA	NA
Tumor size, >5 cm *vs* ≤5 cm	1.574 (0.686-3.608)	0.284	NA	NA
Tumor encapsulation, none *vs* complete	1.233 (0.624-2.437)	0.546	NA	NA
Satellite lesion, yes *vs* no	1.155 (0.355-3.754)	0.811	NA	NA
Vascular invasion, yes *vs* no	2.093 (1.109-3.951)	0.023	2.02 (1.524-3.836)	0.032
Edmondson stage, III/IV *vs* I/II	0.814 (0.386-1.714)	0.588	NA	NA
Preoperative PIVKA-II, >373.5 *vs* ≤373.5 AU/mL	2.965 (1.572-5.595)	0.001	2.877 (1.524-5.429)	0.001

*Clinicopathological variables were adopted for their prognostic significance by univariate analyses; NA, not applicable

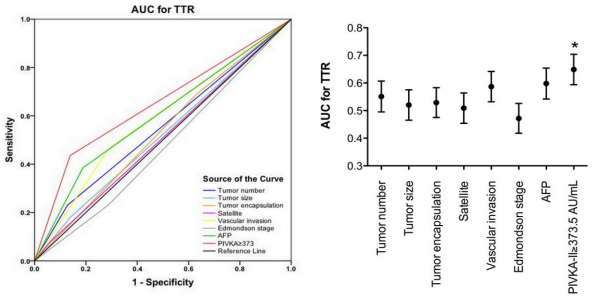

Figure 5. Predictive Ability of PIVKA-II ≥373.5mAU/mL was Compared with Other Clinical Parameters by ROC Curves in 140 BCLC Stage 0-A HCC Patients. The AUC with 95%CI for time to recurrence are also shown. *p<0.05 versus PIVKA-II ≥373.5mAU/mL

373.5 mAU/mL, elevated alanine aminotransferase, AFP, tumor size, tumor encapsulation, satellite lesion, vascular invasion, Edmondson stage, and level of PIVKA-II (Table 2). Level of AFP, liver cirrhosis, vascular invasion, and level of PIVKA-II were unfavorable prognostic variables for recurrence (p<0.05) (Table 2). In multivariate analysis, PIVKA-II 373.5 mAU/mL and vascular invasion (HR, 2.020; 95% CI 1.524-3.836; p=0.032) were independent prognostic factor for TTR, and PIVKA-II 373.5 mAU/mL was the strongest independent prognostic factor (HR, 2.877; 95% CI 1.524-5.429; p=0.001, Table 2).

The prognostic significance of preoperative PIVKA-II 373.5 mAU/mL within clinical subgroups was further investigated. In patients with AFP ≤400 ng/mL, patients with PIVKA-II level ≥ 373.5 mAU/mL had higher recurrence rates (45.5% *vs* 16.7%) and shorter TTR than those with PIVKA-II level <373.5 mAU/mL (p=0.006) (Figure 4A). Patients with preoperative PIVKA-II level ≥ 373.5 mAU/mL showed a relatively higher risk of developing postoperative recurrence than those with PIVKA-II level <373.5 mAU/mL in low-recurrence-risk subgroups, including tumor size ≤5 cm (54.2% *vs* 18.1%; p<0.001), single tumor (56.0% *vs* 19.1%; p<0.001), absence of satellite lesions (53.3% *vs* 19.8%; p<0.001), absence of vascular invasion (52.6% *vs* 14.9%; p=0.002), and Edmondson stage I/II (60.9% *vs* 20.3%; p<0.001), (Figure 4B-G).

ROC analysis showed that the AUC for the level of PIVKA-II was 0.649, with a sensitivity of 43.6% and specificity of 86.1% (p=0.006; 95% CI 0.540-0.757). Compared with other clinical indices, the level of PIVKA-II prior to resection was the strongest factor for predicting early recurrence in HCC (AUCs with 95% CI for TTR; p<0.05 *vs* PIVKA-II 373.5 mAU/mL) (Figure 5). The predictive power of the simplified model was higher than the single factors of level of PIVKA-II and vascular invasion.

Discussion

The most effective therapeutic options for HCC offering a favorable prognosis are hepatectomy and liver transplantation. However, even such curative surgery does not guarantee full recovery, and this failure is owing in large part to the high incidence of recurrence (50-70% at 5 years) (Cha et al., 2003). The most significant reason for the unsatisfactory therapeutic outcome is residual micrometastases formed prior to resection, or dissemination of tumor cells during surgical manipulation (Shah et al., 2007). Unfortunately, routine diagnostic approaches are thus far unable to identify the HCC patient subpopulation at high risk of developing micrometastases preoperatively (Shan et al., 2006). Increasing attention has been poured into developing markers for predicting HCC recurrence risk. For instance, The fibroblast growth factor-inducible 14 (Fn14) expression, closely associated with AFP, can be believed to indicate poor surgical outcome when it is detected overexpressed (Li et al., 2013) . The low counts of γδ T cells in peritumoral liver tissue suggest a higher incidence of recurrence in HCC and are good to postoperative recurrence, especially in patients with early-stage HCC (Cai et al., 2014).

For patients with relevant portal hypertension (5-year survival: 50%, BCLC stage A2) or both adverse prognostic factors (5-year survival: 25%, BCLC stage A3), BCLC is the sole system that links staging to treatment indication. Very early HCC stage (BCLC 0) is defined as patients with well-preserved liver function diagnosed with the carcinoma in situ, which mostly involves a single HCC lesion <2cm. The best candidates for resection are patients

with single asymptomatic HCC with preserved liver function (BCLC 0-A), which may achieve 70% survival rates after treatment (Lovet et al., 1999).

PIVKA-II is an aberrant form of prothrombin produced by HCC cells. Recently, The effects of PIVKA-II on growth and migration of human vascular endothelial cells (HUVECs) was demonstrated (Wang et al., 2009a). They found that PIVKA-II significantly stimulated the proliferation of HUVECs (ECV304 cells) in a dose- and time-dependent manner and expression of epidermal growth factor receptor, vascular endothelial growth factor, and matrix metalloproteinase-2. Anatomical resection appeared to have a beneficial effect on recurrence-free survival after hepatectomy for HCC, and PIVKA-II measurement was effective in predicting HCC recurrence and had the advantage that it can be assessed before surgery (Yamamoto et al., 2010). It was suggested that PIVKA-II is a useful tumor marker for HCC, complementary to AFP (Kim et al., 2007). Serial measurements of both markers after resection might be helpful for early diagnosis of tumor recurrence. Furthermore, combination of PIVKA-II and AFP improves initial diagnosis of HCC, and the sensitivity of these markers is greatest at the time of HCC identification and noticeably less so at earlier time points (Mittal et al., 2012). Our study suggests that PIVKA-II is useful for early diagnosis of BCLC 0-A HCC recurrence. However, our study showed that AFP was only a risk factor (HR, 2.123; 95% CI 1.112-4.053; p=0.023), and not an independent risk factor of BCLC 0-A HCC early recurrence. PIVKA-II 373.5 mAU/mL was the strongest independent prognostic factor (HR, 2.877; 95% CI 1.524-5.429; p=0.001, Table 2). Thus, the preoperative detection of serum PIVKA-II level might serve as a novel indicator reflecting early recurrence of BCLC 0-A HCC. In clinical practice, it is challenging to predict tumor relapse in the lowest-recurrence-risk HCC subgroups (Tung-Ping Poon et al., 2000; Shah et al., 2006). The present study is the first to show that preoperative serum PIVKA-II level retains its prognostic value in subgroups for which conventional clinicopathological variables offer limited prediction of tumor recurrence. So far, AFP level is the most extensively used diagnostic biomarker and tumor recurrence indicator of HCC (Chan et al., 2009). Clinical data demonstrated that low serum AFP concentration (e.g., <400 ng/mL) was associated with better clinical outcome. Nevertheless, it is difficult to monitor recurrence in the 30-40% of HCC patients with low AFP levels (Shan et al., 2006; Wang et al., 2009b). It was demonstrated that HCC patients with low values of both AFP and PIVKA-II had more favorable clinical characteristics and showed better prognosis than those with elevated levels of AFP or PIVKA-II (Kang et al., 2012). Combination of AFP and PIVKA-II response has predictive power for PFS and overall survival comparable to radiological criteria and better than AFP response alone (Park et al., 2013). Here, we showed that determination of preoperative serum PIVKA-II level is a promising and feasible marker for prediction of recurrence in patients with low AFP concentration (p=0.006). Large cohort studies should be undertaken to validate the prognostic significance in this specific HCC patient subpopulation. Furthermore, in clinicopathologically lower-risk patients (tumor size ≤ 5cm, single tumor, no satellite lesion, no vascular invasion, Edmondson stage I/II, and BCLC stage 0-A), those with serum PIVKA-II <373.5 mAU/mL postoperatively showed lower recurrence risk than those with PIVKA-II ≥ 373.5 mAU/mL (p≤0.002). We propose that determination of serum PIVKA-II level may provide a powerful test enabling accurate and early decision making to tailor the most effective therapy according to characteristics of individual tumors.

The limitations of this study were its relatively small cohort size, short follow-up time, and data from a single study center. Most patients with HCC in China have hepatitis B and the predictive model needs validation in patients with HCC from those areas. Additionally, the findings need a larger population prospective study to validate their usefulness.

To the best of our knowledge, this is the first study to identify the levels of PIVKA-II in BCLC 0-A patients. BCLC 0-A HCC is generally associated with good prognosis. Preoperative level of PIVKA-II >373.5 mAU/mL is an independent prognostic indicator for recurrence in BCLC 0-A HCC patients undergoing curative resection. In patients with low AFP level, PIVKA-II will improve the finding of early recurrence of HCC.

Acknowledgements

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