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Validation of the OncoHepa test, a multigene expression profile test, and the tumor marker-volume score to predict postresection outcome in small solitary hepatocellular carcinomas

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Purpose: OncoHepa test is a multigene expression profile test developed for assessment of hepatocellular carcinoma (HCC) prognosis. Multiplication of α-FP, des-γ-carboxy prothrombin (DCP) and tumor volume (TV) gives the α-FP-DCP-volume (ADV) score, which is also developed for assessment of HCC prognosis.
Methods: The predictive powers of OncoHepa test and ADV score were validated in 35 patients who underwent curative hepatic resection for naïve solitary HCCs ≤5 cm.
Results: Median tumor diameter was 3.0 cm. Tumor recurrence and patient survival rates were 28.6% and 100% at 1 year, 48.6% and 82.9% at 3 years, and 54.3% and 71.4% at 5 years, respectively. The site of first tumor recurrence was the remnant liver in 18, lung in 1, and the peritoneum in 1. All patients with HCC recurrence received locoregional treatment. OncoHepa test showed marginal prognostic significance for tumor recurrence and patient survival. ADV score at 4log also showed marginal prognostic difference with respect to tumor recurrence and patient survival. Combination of these 2 tests resulted in greater prognostic significance for both tumor recurrence (P = 0.046) and patient survival (P = 0.048).
Conclusion: Both OncoHepa test and ADV score have considerably strong prognostic power, thus individual and combined findings of OncoHepa test and ADV score will be helpful to guide postresection surveillance in patients with solitary HCCs ≤5 cm.
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Key Words: Recurrence, Survival, Prognosis, Genes

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies around the world, and thus one of the leading causes of cancer-related death [1]. In patients with small, solitary HCCs, various locoregional treatments are indicated, but hepatic resection (HR) is still considered the first-line therapy in patients with preserved hepatic function [2,3]. However, tumor recurrence frequently develops following curative HR for small solitary HCCs, and the risk of recurrence is greater after HR for multiple or large HCCs. There are various risk factors influencing the postresection prognosis

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of HCC, but with respect to small solitary HCCs specifically, only a few clinicopathological factors, such as microvascular invasion (MVI), are known to be of limited value [4-6]. Thus, it is clinically important to identify prognostic factors that are reliably applicable to HR for small solitary HCCs.

The OncoHepa test (CbsBioscience Inc., Daejeon, Korea), a multigene expression profile test, was developed to assess the risk of tumor recurrence and death in patients with HCC. After validation through a multicenter study [7], it was licensed as a new Health Technology by the Ministry of Health and Welfare in Korea in 2010. Another prognostic prediction model, the ADV score, in which the levels of expression of 2 tumor markers—α-FP and des-γ-carboxy prothrombin (DCP)—are multiplied by the tumor volume (TV), was also developed to quantify the biological aggressiveness of HCC and to predict postresection prognosis [3].

Although these 2 tests are applicable to HCCs of any size, in this study, we focused on validation of their predictive power in patients who underwent curative HR for naïve, solitary HCCs of ≤5 cm.

METHODS

Patient selection

Our institutional liver cancer surgery database was searched extensively to identify patients who underwent macroscopic curative resection for naïve solitary HCCs ≤5 cm between January 2010 and December 2011. After matching with the HCC patient list at the Bio-Resource Center at our institution, 35 patients were identified whose HCC tissues were stored in a fresh-frozen state at -80°C.

These 35 patients were enrolled in this study, and their fresh-frozen HCC tissues were examined by OncoHepa test. Their medical records were retrospectively reviewed after approval by the Institutional Review Board of Asan Medical Center (IRB 2017-0575). Patients were followed until March 2017 through a review of institutional medical records and National Health Insurance Service records, resulting in a follow-up period of ≥7 years, or until patient death.

Preoperative evaluation, surgical procedures, and follow-up

The preoperative imaging evaluation for HCC included dynamic abdomen and pelvis CT, chest CT, and MRI. The hepatic functional reserve was assessed using the indocyanine green retention rate at 15 minutes and the evidence of portal hypertension on imaging and endoscopic studies. The extent of HR was determined by the proportion of the future liver remnant volume after consideration of tumor-free resection margins and the hepatic functional reserve. Perioperative evaluation, perioperative follow-up, and treatment for tumor recurrence were described previously [2,3,8-10].

OncoHepa test

The OncoHepa test was performed with the fresh-frozen HCC tissue samples stored at the Bio-Resource Center at our institution. This study assesses the signature of 4 genes (*CDH1*, *ID2*, *MMP9*, and *TCF3*) by quantitative real-time polymerase chain reaction (PCR) [7].

RNA extraction and cDNA synthesis

Total RNA was extracted from HCC tissues using the RNeasy minikit (Qiagen, Hilden, Germany). Samples containing 4 μg of total RNA were incubated with 2 μL of 10 lM oligo $d(T)_{18}$ primer (Genotech, Daejeon, Korea) at 70°C for 7 minutes and cooled on ice for 5 minutes. After adding the enzyme mix to the annealed total RNA sample, the reaction was incubated for 90 minutes at 42°C prior to heat inactivation of the reverse transcriptase at 80°C for 10 minutes. The cDNA samples were brought up to a final volume of 400 μL with the addition of diethylpyrocarbonate-treated water.

Quantitative real-time PCR

Using Applied Biosystems Prism 7900HT instruments (Applied Biosystems, Foster City, CA, USA), the real-time PCR analysis was performed in a total volume of 10 μL with the following amplification steps: an initial activation step at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds and elongation at 60°C for 1 minute. The primer and probe sequences were designed using Primer Express 3.0 software (Applied Biosystems) (Table 1), and all probe sequences were labeled with FAM at the 5' end and with TAMRA at the 3' end. The mRNA levels of 4 target genes (*CDH1*, *ID2*, *MMP9*, and *TCF3*) were measured in triplicate and and then normalized relative to a set of reference

Table 1. Oligonucleotide sequences of polymerase chain reaction primers and probes

Gene	Sequence
CDH1	Forward: 5′-AAA TCT GAA AGC GGC TGA TAC TG-3′
	Reverse: 5′-CGG AAC CGC TTC CTT CAT AG-3′
	Probe: 5′-CCC CAC AGC CCC GCC TTA TGA-3′
ID2	Forward: 5′-AAC GAC TGC TAC TCC AAG CTC AA-3′
	Reverse: 5′-GGA TTT CCA TCT TGC TCA CCT T-3′
	Probe: 5′-TGC CCA GCA TCC CCC AGA ACA A-3′
MMP9	Forward: 5′-GGG CTC CCG TCC TGC TT-3′
	Reverse: 5′-ACT CCT CCC TTT CCT CCA GAA C-3′
	Probe: 5′-TGC CAT GTA AAT CCC CAC TGG GAC C-3′
TCF3	Forward: 5′-GCT GCC TTT GGT CTC TGG TTT-3′
	Reverse: 5′-AGA AAT GCA ATG CTC AGT CTA GGA-3′
	Probe: 5′-AGT CCC GTG TCT CTC GCT ATT TCT GCT G-3′

genes beta-2-microglobulin, glyceraldehyde 3-phosphate dehydrogenase, hydroxymethylbilane synthase, hypoxanthine phosphoribosyltransferase 1, and succinate dehydrogenase complex flavoprotein subunit A, by subtracting the average of the expression of the 5 reference genes as an internal control. Details of these procedures were described previously [7,11,12].

Calculation of risk score

Risk score was derived by the summation of each gene expression level multiplied by its corresponding coefficient,

Table 2. Comparison of the clinicopathological characteristics in the low-risk group and the high-risk group according to the OncoHepa test

Parameter	Low-risk group (n = 28)	High-risk group (n = 7)	P-value
Age (yr)	54.0 ± 6.3	54.4 ± 7.8	0.55
Sex, male:female	21:7	6:1	>0.99
HBs Ag (+)	28 (100)	6 (85.7)	0.20
HCV Ab (+)	0 (0)	1 (14.3)	>0.99
ICG-R15 (%)	14.9 ± 4.9	16.3 ± 5.7	0.47
Liver function test			
Albumin (g/dL)	3.3 ± 0.7	3.3 ± 0.7	0.94
Total bilirubin (mg/dL)	1.2 ± 0.4	1.2 ± 0.5	0.37
Prothrombin time (INR)	1.2 ± 0.2	1.2 ± 0.2	0.71
Platelet count ($10^3/\mu L$)	141 ± 34	157 ± 25	0.55
α-FP (ng/mL)			
Mean	303.3 ± 885.2	160.2 ± 264.3	
Median	19.1	14.8	0.42
DCP (mAU/mL)			
Mean	729.3 ± 1612.9	162.4 ± 334.8	
Median	50	31	0.14
Liver cirrhosis	22 (78.6)	7 (100)	0.31
Type of hepatic resection			>0.99[a]
Right hepatectomy	2 (7.1)	1 (14.3)	
Left hepatectomy	1 (3.6)	1 (14.3)	
Right anterior sectionectomy	8 (28.6)	2 (28.6)	
Right posterior sectionectomy	8 (28.6)	1 (14.3)	
Left lateral sectionectomy	1 (3.6)	0 (0)	
Left medial sectionectomy	1 (3.6)	0 (0)	
Partial hepatectomy	7 (25.0)	2 (28.6)	
Tumor size (cm)			
Mean	3.4 ± 1.0	3.3 ± 1.0	0.60
Median	3.0	3.0	
Tumor volume (mL)			
Mean	25.3 ± 20.0	23.6 ± 17.9	0.38
Median	14.1	14.1	
Microvascular invasion	3 (10.7)	0 (0)	>0.99
Edmondson-Steiner tumor differentiation (n)			
Worst, I–II:III–IV	6:22	3:4	0.34
Most, I–II:III–IV	15:13	4:3	>0.99
ADV score (log_{10})			
Mean	4.7 ± 1.6	4.3 ± 1.5	0.94
Median	4.5	3.8	
Tumor stage			
7th AJCC, T1:T2	25:3	1:6	>0.99
8th AJCC, T1a:T1b:T2	4:21:3	1:6:0	>0.99
BCLC, 0:A	2:26	1:6	0.50

Values are presented as mean ± standard deviation or number (%).
ICG-R15, indocyanine green retention rate at 15 minutes; DCP, des-γ-carboxy prothrombin; AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; ADV score, multiplication of α-FP, DCP, and tumor volume expressed in log_{10}.
[a]Anatomical resection *vs.* partial hepatectomy.

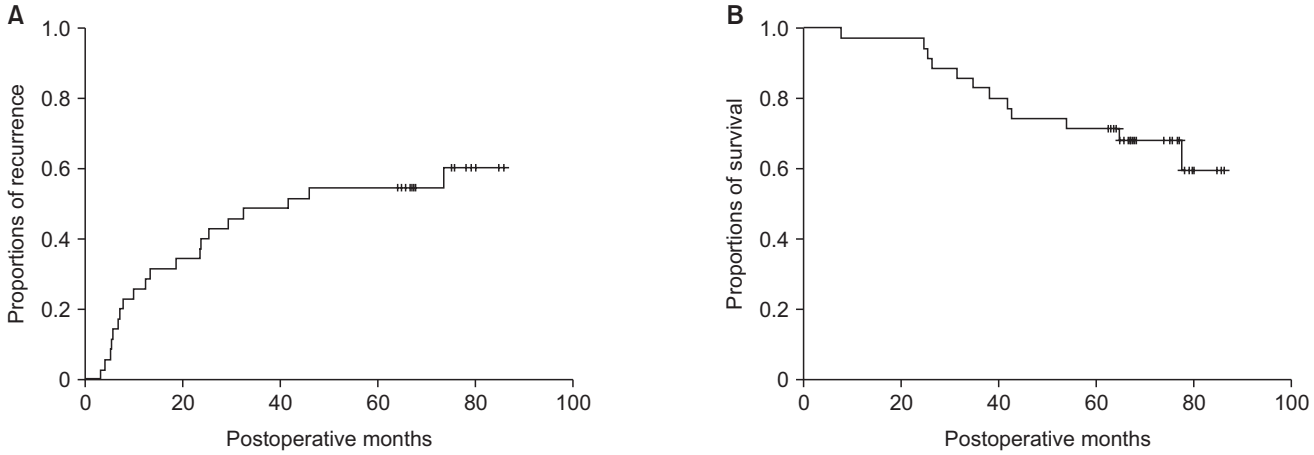

Fig. 1. Postresection cumulative tumor recurrence (A) and overall patient survival (B).

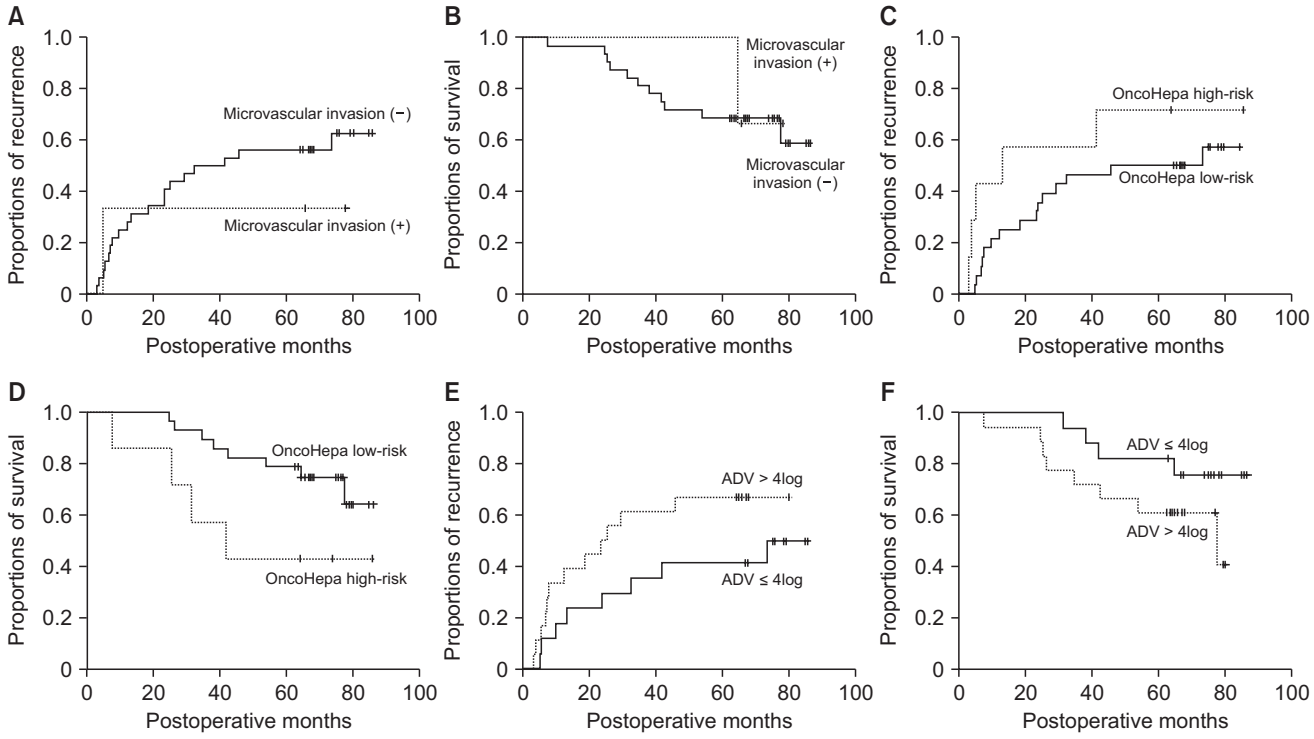

Fig. 2. Postresection cumulative tumor recurrence (A, C, E) and overall patient survival (B, D, F) according to microvascular invasion (A, B), the OncoHepa test (CbsBioscience Inc., Daejeon, Korea) (C, D), and the ADV score (E, F).

Table 3. Multivariate analyses of the risk factors associated with tumor recurrence and patient survival

Parameter	Tumor recurrence			Patient survival		
	HR	95% CI	P-value	HR	95% CI	P-value
OncoHepa test (high risk *vs.* low risk)	2.2	0.9–6.2	0.098	4.1	1.2–14.3	0.025
ADV score (>4log *vs.* ≤4log)	2.2	0.9–5.5	0.095	3.1	0.9–10.9	0.062

HR, hazard ratio; CI, confidence interval.

as follows: risk score = (-0.333 × CDH1) + (-0.400 × ID2) + (0.339 × MMP9) + (0.387 × TCF3), wherein *CDH1, ID2, MMP9,* and *TCF3* refer to the $\log_2$-transformed and normalized results for each gene. The risk score was used to stratify the patients into high-risk (>0.303) or low-risk (<0.303) groups. High risk indicates poor patient survival [7].

ADV score integrated with tumor markers and TV

Multiplication of the α-FP (ng/mL), the DCP level (or protein-induced by vitamin K absence or antagonist-II; mAU/mL) and TV (mL) gives the ADV score, which is expressed on the logarithmic scale ($\log_{10}$) [3,10]. The ADV score was developed to quantify the biological aggressiveness of HCC and to predict postresection prognosis of HCC; its cutoff for post-resection prognosis of small HCC was set to 4log.

Statistical analysis

Continuous variables were analyzed by using the Student t-test or analysis of variance, depending on the distribution. Incidence variables were compared by the chi-square test or Fisher exact test. Survival curves were estimated by the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards regression analysis was used to obtain the hazard ratio and 95% confidence interval. Statistical significance was set at $P < 0.05$, and in certain analyses, P-values between 0.05 and 0.10 were considered to be marginally significant after consideration of the small sample number. IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA) was used for statistical analyses.

RESULTS

Clinicopathological features

The clinical and pathological features of the 35 study patients are summarized in Table 2. HBV infection was present in 34 patients (96.1%). No patient underwent any HCC treatment before HR. Preoperative liver function tests showed no difference between the OncoHepa low- and high-risk groups.

The types of HR were anatomical resection in 28 patients (80.0%) and nonanatomical partial hepatectomy in 7 patients (20.0%). The median tumor diameter was 3.0 cm (range, 1.7–5.0 cm), and the median TV was 14.1 mL (range, 2.6–65.4 mL). Pathological study revealed that MVI was present only in 3 patients (8.6%). No patient showed evidence of macrovascular invasion or satellite nodules. Liver cirrhosis was present in 29 patients (82.9%).

Tumor recurrence and patient survival

There was no perioperative mortality within 3 months. During the mean follow-up period of 61.1 ± 20.5 months (range, 7.6–86.2 months), tumor recurrence was identified in 20 patients (57.1%). Prognostic analysis indicated that the cumulative 1-, 3-, and 5-year tumor recurrence rates were 28.6%, 48.6%, and 54.3%, respectively (Fig. 1A). All-cause death occurred in 12 patients (34.3%), all of whom died from HCC recurrence. The 1-, 3-, and 5-year overall patient survival rates were 100%, 82.9%, and 71.4%, respectively (Fig. 1B).

The site of the first tumor recurrence was the remnant liver in 18 patients, the lung in 1 patient, and the peritoneum in 1 patient. All patients with HCC recurrence received locoregional treatment including transarterial chemoembolization (n = 16), radiofrequency ablation (n = 1), resection of the pulmonary metastasis (n = 1), and peritoneal seeding (n = 1).

Risk factor analysis for tumor recurrence and patient survival

Univariate analyses for tumor recurrence and patient survival were performed according to MVI, OncoHepa test results, and the ADV score.

The cumulative 5-year tumor recurrence rate was 56.2% in 32 MVI-negative patients and 33.3% in 3 MVI-positive patients (P = 0.55, Fig. 2A). The cumulative 5-year patient survival rate was 68.8% in 32 MVI-negative patients and 100% in 3 MVI-positive patients (P = 0.81, Fig. 2B).

The cumulative 5-year tumor recurrence rate was 50.0% in 28 OncoHepa low-risk patients and 71.4% in 7 OncoHepa high-risk patients (P = 0.097, Fig. 2C). The cumulative 5-year patient survival rate was 78.6% in 28 OncoHepa low-risk patients and 42.9% in 7 OncoHepa high-risk patients (P = 0.048, Fig. 2D).

The cumulative 5-year tumor recurrence rate was 41.2% in 17 ADV score ≤4log patients and 66.7% in 18 ADV score >4log patients (P = 0.098, Fig. 2E). The cumulative 5-year patient survival rate was 82.4% in 17 ADV score ≤4log patients and 61.1% in 18 ADV score >4log patients (P = 0.096, Fig. 2F).

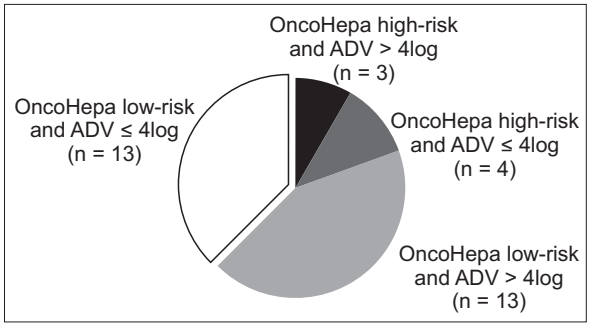

Fig. 3. Proportions of the patients in the indicated groups according to the OncoHepa test (CbsBioscience Inc., Daejeon, Korea) and the ADV score. The left-sided group indicates the combined low-risk group and the sum of the three right-sided groups indicates the combined high-risk group.

Combination of the OncoHepa test and the ADV score

Because univariate analyses using OncoHepa findings or the ADV score with a 4log cutoff showed marginally statistically significant differences, we considered that these parameters might be independent prognostic factors and performed multivariate analyses for tumor recurrence and patient survival (Table 3). Thus, the OncoHepa findings and ADV score were

Table 4. Comparison of the clinicopathological characteristics in the combined low-risk group and the combined high-risk group according to the OncoHepa test finding and ADV score with 4log cutoff

Parameter	Combined low-risk group (n = 13)	Combined high-risk (n = 22)	P-value
Age (yr)	53.9 ± 5.8	54.2 ± 7.0	0.42
Sex, male:female	11:2	16:6	0.68
HBs Ag (+)	13 (100)	21 (95.5)	>0.99
HCV Ab (+)	0 (0)	1 (4.5)	>0.99
ICG-R15 (%)	13.9 ± 4.5	16.0 ± 5.3	0.45
Liver function test			
Albumin (g/dL)	3.5 ± 0.6	3.2 ± 0.7	0.73
Total bilirubin (mg/dL)	1.1 ± 0.4	1.3 ± 0.5	0.28
Prothrombin time (INR)	1.1 ± 0.1	1.2 ± 0.2	0.098
Platelet count (10^3/μL)	147 ± 35	142 ± 32	0.58
α-FP (ng/mL)			
Mean	7.5 ± 8.6	432.6 ± 981.1	
Median	4.1	41.6	0.028
DCP (mAU/mL)			
Mean	52.2 ± 61.2	949.1 ± 1,774.6	
Median	33	60	0.002
Liver cirrhosis (n)	11 (84.6)	18 (81.8)	>0.99
Type of hepatic resection (n)			0.72[a]
Right hepatectomy	1 (7.7)	2 (9.1)	
Left hepatectomy	0 (0)	2 (9.1)	
Right anterior sectionectomy	4 (30.8)	6 (27.3)	
Right posterior sectionectomy	3 (23.1)	6 (27.3)	
Left lateral sectionectomy	0 (0)	1 (4.5)	
Left medial sectionectomy	1 (7.7%)	0 (0)	
Partial hepatectomy	4 (30.8)	5 (22.7)	
Tumor size (cm)			
Mean	3.0 ± 1.0	3.6 ± 1.0	0.097
Median	2.7	3.5	
Tumor volume (mL)			
Mean	18.3 ± 16.8	28.9 ± 20.1	0.22
Median	10.3	22.4	
Microvascular invasion	2 (15.4)	1 (4.5)	0.55
Edmondson-Steiner tumor differentiation			
Worst, I–II:III–IV	4:9	5:17	0.70
Most, I–II:III–IV	9:4	10:12	0.29
ADV score (log_{10})			
Mean	2.3 ± 0.5	5.4 ± 1.4	0.010
Median	3.3	5.5	
Tumor stage			
7th AJCC, T1:T2	11:2	21:1	0.54
8th AJCC, T1a:T1b:T2	3:8:2	2:19:1	0.54
BCLC, 0:A	2:11	1:21	0.54

Values are presented as mean ± standard deviation or number (%).
ICG-R15, indocyanine green retention rate at 15 minutes DCP, des-γ-carboxy prothrombin AJCC, American Joint Committee on Cancer BCLC, Barcelona Clinic Liver Cancer ADV score, multiplication of α-FP, DCP, and tumor volume expressed in log_{10}.
[a]Anatomical resection *vs.* partial hepatectomy.

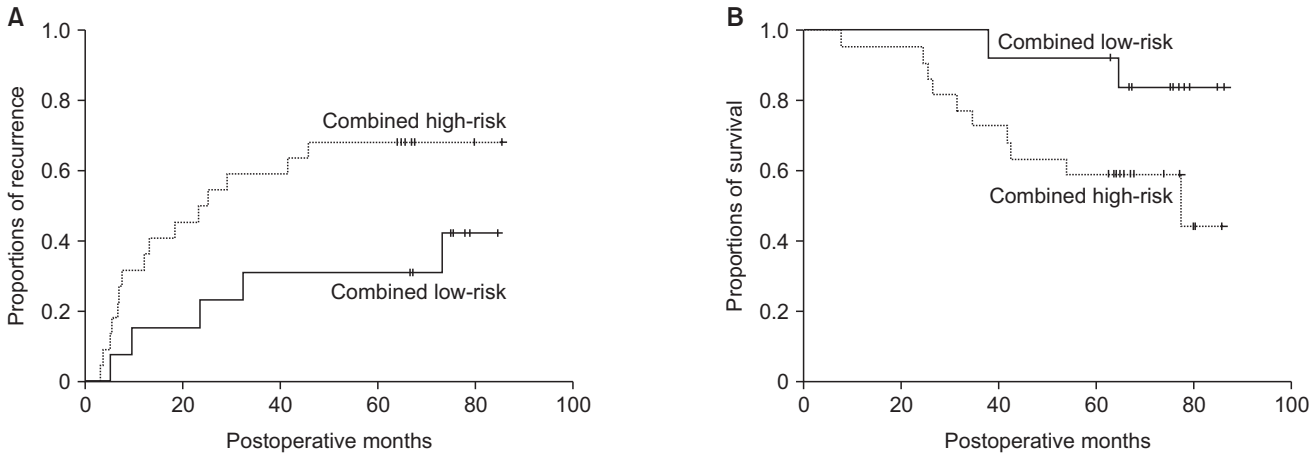

Fig. 4. Postresection cumulative tumor recurrence (A) and overall patient survival (B) according to the combined risk, determined from the combination of the OncoHepa test (CbsBioscience Inc., Daejeon, Korea) and the ADV score.

combined, and patients were divided into 2 groups: the combined high-risk group (OncoHepa high-risk or ADV score >4log) and the combined low-risk group (OncoHepa low-risk and ADV score ≤4log) (Fig. 3). The clinical and pathological features of study patients belonged to the combined low- and high-risk groups are summarized in Table 4, in which significant difference was present in α-FP, DCP, and ADV score.

The cumulative 5-year tumor recurrence rate was 30.8% in 13 combined low-risk patients and 68.2% in 22 combined high-risk patients (P = 0.046, Fig. 4A). The cumulative 5-year patient survival rate was 92.3% in 13 combined low-risk patients and 59.1% in 22 combined high-risk patients (P = 0.048, Fig. 4B).

DISCUSSION

HCC is a unique disease with highly heterogeneous clinicopathological features, and is thus difficult to treat effectively and to predict prognosis reliably. Tumor size is one of the most important prognostic factors following HR in HCC. We previously demonstrated that the post-resection prognosis gradually worsens according to an incremental increase in HCC size, from 1 cm to 10 cm, regardless of the MVI status [2]. In solitary HCCs, the incidence of MVI was 31% in HCCs ≤5 cm, 41% in HCCs of 5.1–6.5 cm, and 58% in HCCs >6.5 cm [13]. We also showed that the incidence of MVI progressively increases with size, from 4.1% in HCCs <2 cm to 13.1% in HCCs of 2.1–4.0 cm, 20.6% in HCCs of 4.1–5.9 cm, 31.5% in HCCs of 6.1–7.9 cm, and 30.7% in HCCs of 8.1–9.9 cm [2]. In the present study, MVI was present in only 8.6% (3 of 35) of patients with solitary HCCs ≤5 cm probably due to intentional selection of small-sized tumors. Because of its low incidence and lower prognostic impact in small-sized HCCs, MVI often cannot be used as an independent prognostic factor in patients with small HCCs [4,10]. Therefore it is necessary to identify other prognostic factors applicable to small HCCs.

The OncoHepa test is a multi-gene expression profile test for risk stratification that uses a cutoff value obtained from a high-volume multicenter study [7]. The four genes used in the OncoHepa test are associated with epithelial-mesenchymal transition (EMT), which plays a critical role in epithelial cancer progression [14]. During development of the OncoHepa test, 12 genes related to EMT process were assessed and then a prognostic 4-gene signature was constructed through training and validation studies [7].

The 4 genes used in the OncoHepa test are associated with tumor invasion and metastasis. E-cadherin (*CDH1*) is a prominent epithelial marker, as the main component of adherent junctions [14] Lower expression of E-cadherin was reported to be associated with poor prognosis [15]. ID2 belongs to the helix-loop-helix family of proteins and represses EMT induced by transforming growth factor-β in epithelial cells [16]. Lower ID2 expression was correlated with higher recurrence in HCC patients [17], and with dedifferentiation of HCC [18]. Matrix metalloproteinases (MMPs) are reported to be upregulated in cells that have undergone EMT as well as in cells capable of inducing EMT [19,20]. Overexpression of MMP9 was linked to the growth of small HCCs [21,22] and correlated with poor prognosis [21-24]. In addition, the expression of transcription factor 3 (TCF3) is reported to be associated with prognosis [7]. TCF3 and Twist are potent repressors of E-cadherin expression [25,26].

The present study suggests that the OncoHepa test is clinically useful for predicting risk of tumor recurrence and patient survival after HR of solitary HCCs ≤5 cm. Its predictive power was greater than that of other established prognostic factors, including MVI, although it showed marginal statistical significance due to the small sample number. In fact, the concept of gene expression signatures for predicting cancer

prognosis is no longer unique. Multiple gene expression signatures have been developed for predicting the prognosis of breast cancers, and they are already included as essential parts of the treatment guidelines for breast cancers [27,28].

Only a very small amount of HCC tissue is necessary for the OncoHepa test; thus, a very thin thread of HCC tissue obtained from a percutaneous liver biopsy can be a suitable specimen. The present study and previous validation studies of the OncoHepa test have used fresh-frozen HCC tissues, which were obtained during HR and preserved at -80°C. This test uses RNA extracted from fresh-frozen HCC tissue, thus HCC tissues preserved in paraffin-embedded blocks may not be suitable probably due to the difficulty of reliable RNA extraction [29,30]. Thus, it is practical to perform this test at the time HCC specimens are prepared for pathological examination after HR.

The ADV score is another prognostic parameter reflecting the tumor biology. Its suitability for use in clinical practice was demonstrated in our previous studies [3,10]. The results of the present study also suggest that the ADV score with a cutoff at 4log is a useful predictor of the risk of tumor recurrence and patient survival after HR of solitary HCCs ≤5 cm. Its predictive power was comparable to that of the OncoHepa test, although it also showed a marginal statistical significance due to the small sample number. Multivariate analyses revealed that these 2 parameters can be independent prognostic factors. Therefore, we combined the two to create a new prognostic prediction model. If any of 2 parameters is high risk, the patient is considered to have a combined high risk. Combined high-risk patients showed significantly inferior outcomes compared to combined low-risk patients. Thus, we think that this combined risk model will be useful to predict patients with a truly low risk of tumor recurrence and patient survival. If the 2 test parameters are available, the combined prognostic prediction power will be greatly enhanced. If only one parameter is available, its prognostic predictive power is still strong enough to be clinically valid.

The present study had some limitations of note. This was a small-volume, single-center study in a HBV-endemic country. Hence, it will be necessary to validate our results in multiple centers and in other geographic regions with different background liver diseases. Another limitation of the present study was that we selected only patients with solitary, small HCCs to avoid bias from inevitable confounding variables.

In conclusion, our results support previous reports that both the OncoHepa test and the ADV score have considerably strong prognostic power in patients who underwent HR of solitary HCCs ≤5 cm. Therefore, the individual and combined findings of the OncoHepa test and the ADV score are helpful to guide postresection surveillance for tumor recurrence and to predict patient survival.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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