World J Surg (2015) 39:1134–1141
DOI 10.1007/s00268-014-2806-6

World Journal of Surgery

ORIGINAL SCIENTIFIC REPORT

The Influence of Histological Differentiation Grade on the Outcome of Liver Resection for Hepatocellular Carcinomas 2 cm or Smaller in Size

Kazunari Sasaki · Masamichi Matsuda · Yu Ohkura · Yusuke Kawamura · Masafumi Inoue · Masaji Hashimoto · Kenji Ikeda · Hiromitsu Kumada · Goro Watanabe

Published online: 7 October 2014

Abstract

Background Small hepatocellular carcinomas (HCC) with poorly differentiated components (PDC) are reportedly at risk of dissemination and needle tract seeding after percutaneous radiofrequency ablation, although it is the preferred treatment for HCC $\leq$ 2 cm because of the low rate of vascular invasion. On the other hand, the clinical outcomes after hepatectomy for these tumors are still unclear because of their rarity.

Methods A total of 233 cases of solitary HCC $\leq$ 2 cm were retrospectively reviewed and divided into two groups according to the presence of PDC: 199 without PDC (NP-HCCs) and 34 with PDC (P-HCCs). The clinicopathological characteristics and prognosis were compared.

Results A comparison of clinicopathological characteristics showed that the elevation of the tumor markers alpha-fetoprotein (AFP) (>20 ng/mL) and des-gamma-carboxyprothrombin (DCP) (>40 AU/L) was significantly frequent in P-HCCs. The 3- and 5-year recurrence-free survival rates for P-HCCS were 39 and 29 %, respectively, which were significantly worse than those for NP-HCCs (64 and 50 %, respectively) ($p < 0.01$). Initial recurrence of P-HCCs was significantly more frequent, as well as extrahepatic recurrence and advanced recurrence in the early period after the operation. Recurrences with tumor dissemination were observed in 15 % of P-HCCs and 4 % of NP-HCCs ($p = 0.03$).

Conclusion PDC is present in 15 % of HCC < 2 cm and should be suspected when the both tumor markers are elevated. Moreover, significantly worse post-hepatectomy outcomes such as early advanced recurrence or recurrence with dissemination should be taken into account if PDC is present even in HCCs $\leq$ 2 cm.

Introduction

Classic hepatocellular carcinoma (HCC) is known to develop in a multistep fashion. Well-differentiated areas are gradually replaced by less well-differentiated tissue when the tumor size reaches a diameter of approximately 2 cm [1, 2]. It is now possible to detect small HCCs because of advances in imaging and establishment of screening guidelines for patients with a high risk of HCC, resulting in an increasing number of early HCC that

K. Sasaki (✉) · M. Matsuda · Y. Ohkura · M. Hashimoto · G. Watanabe
Department of Digestive Surgery, Hepatopancreatobiliary Surgery Unit, Toranomon Hospital, Tokyo, Japan
e-mail: sasakikazunari1978@hotmail.com

Y. Kawamura · K. Ikeda · H. Kumada
Department of Hepatology, Toranomon Hospital, Tokyo, Japan

M. Inoue
Department of Pathology, Toranomon Hospital, Tokyo, Japan

undergo treatment [3]. In these situations, surgeons may encounter cases of resected HCCs of a small size (2 cm or smaller) that have poorly differentiated components (PDC). The carcinogenesis process of these tumors deviates greatly from the well-known multistep development theory.

The HCCs that have PDCs are reportedly at risk of dissemination, needle tract tumor seeding, and intrahepatic dissemination after percutaneous radiofrequency ablation (RFA). Based on these observations, several reports recommend hepatectomy rather than RFA for HCCs that contain PDCs and pre-treatment detection of PDCs in HCCs has attracted attention [4–6]. However, the influence of PDCs in small HCCs on outcomes after hepatectomy and the characteristics of these tumors remain unclear.

The aim of the present study was to examine the clinicopathological characteristics of small HCCs $\leq$ 2 cm with PDC, and to determine whether the outcome of hepatectomy is influenced by the histologic grade in small HCCs $\leq$ 2 cm.

Methods

Patients

A total of 649 cases that underwent primary curative hepatectomy for HCC (R0 or R1 resection) between 1994 and 2012 were retrospectively reviewed. Of the 649 cases, 246 had solitary tumors $\leq$2 cm in size and were examined in detail. Cases with solitary tumors were chosen to enable accurate assessment of the influence of histologic grade on operative outcomes. Ten cases with an uncertain histologic grade due to total necrosis secondary to preoperative transcatheter chemoembolization were excluded, as well as three cases with unclear outcomes 1 year after primary resection. Thus, 233 cases of solitary HCC $\leq$ 2 cm were analyzed in this study, which were followed up until death or December 2013. The clinicopathological characteristics of these cases are shown in Table 1, including the number and size of tumors, width of the surgical margin, and presence of liver cirrhosis.

Table 1 Comparison of clinicopathological characteristics between differentiation grades

Differentiation grade[a]	Non-poorly		Poorly $N = 34$	Non-poorly versus poorly
	Well $N = 54$	Moderately $N = 145$		
(a) Patient-related factors				
Male sex % (yes/no)	**72** (39/15)	**75** (109/36)	**79** (27/7)	0.67
Age > 60 years % (yes/no)	**67** (36/18)	**48** (70/75)	**53** (18/16)	1.00
HBV infection positive % (yes/no)	**22** (12/42)	**36** (52/93)	**29** (10/24)	0.84
HCV infection positive % (yes/no)	**70** (38/16)	**57** (82/63)	**62** (21/13)	1.00
(b) Background liver-related factors				
Platelet count $<10^5$ (μL) % (yes/no)	**46** (25/29)	**34** (49/96)	**35** (12/22)	1.00
Child-Pugh grade B % (yes/no)	**22** (12/42)	**8** (12/133)	**12** (4/30)	1.00
Liver cirrhosis % (yes/no)	**70** (38/16)	**66** (95/50)	**79** (27/7)	0.17
(c) Pre-operative tumor markers				
AFP > 100 ng/mL % (yes/no)	**11** (6/48)	**20** (29/116)	**26** (9/25)	0.24
DCP > 100 AU/L %[b] (yes/no)	**2** (1/51)	**12** (17/123)	**15** (5/28)	0.35
Elevation of both tumor markers[b,c] (yes/no)	**4** (2/50)	**10** (14/126)	**27** (9/24)	*<0.01*
(d) Operative factors				
Anatomical resection % (yes/no)	**5** (2/52)	**17** (24/121)	**24** (8/26)	0.12
Surgical margin >5 mm % (yes/no)	**46** (25/29)	**54** (78/67)	**47** (16/18)	0.71
(e) Tumor-related factors				
Non-boundary macroscopic appearance % (yes/no)	**22** (12/42)	**39** (56/89)	**47** (16/18)	0.18
Capsule formation % (yes/no)	**37** (20/34)	**72** (104/41)	**71** (24/10)	0.44
Microscopic vascular invasion % (yes/no)	**9** (5/49)	**18** (26/119)	**24** (8/26)	0.32

HBV hepatitis B virus, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxyprothrombin

[a] Differentiation grade was determined by the differentiation component with the worst grade in the entire specimen

[b] DCP was not measured in six patients

[c] Concurrent elevation of alpha-fetoprotein (>20 ng/mL) and des-gamma-carboxyprothrombin (>40 AU/L)

Treatment selection

The indications for hepatectomy were basically compatible with the recommendations of the Consensus-Based Clinical Practice Manual proposed by the Japan Society of Hepatology [7]. In our institution, local ablation such as RFA is generally recommended for solitary HCCs $\leq$ 2 cm. Local ablation treatments as curative treatment had been indicated in 352 cases of HCCs $\leq$ 2 cm during the study period. Hepatectomy was indicated for cases with preserved liver function with the HCC located peripherally, in the subphrenic space, or near major vasculature. Anatomical resection (AR) was defined as resection of liver segments according to the territories supplied by the portal vein. AR was performed for tumors located centrally or close to major vessels if appropriate, while limited non-AR was preferred for tumors located peripherally or if extrahepatic growths were present. In addition, AR was indicated for tumors with a high risk of spread such as those with "a heterogeneous enhancement pattern with irregular ring-like structures" on three-phase-enhanced CT, and high tumor marker levels, regardless of tumor size [8]. The width of the surgical margin was principally 5 mm because previous studies showed that small tumors seldom have microsatellite lesions greater than 5 mm [9–11]. None of the patients received liver transplantation for HCC $\leq$ 2 cm during the study period.

Histopathologic examination and definition of clinical variables

Two experienced pathologists examined the resected specimens. Variables were defined according to "the general rule for the clinical and pathological study of primary liver cancer" determined by the Liver Cancer Study Group of Japan, and the pathological classification system of the World Health Organization [12, 13]. PDC was defined according to the aforementioned pathological guidelines, as follows: "PDC proliferate in a solid pattern without distinct sinusoid-like blood spaces, and only slit-like blood vessels are observed in large tumor nests. Neoplastic cells show an increased nuclear/cytoplasmic ratio and frequent pleomorphism, including bizarre giant cells" [13]. Only four patients had micrometastases around the main tumor.

The macroscopic appearance of the HCC was divided into two groups based on the classification of the aforementioned guidelines: boundary type, which included the vaguely nodular and single nodular type, and non-boundary type, which included the single nodular with extranodular growth, the confluent multinodular, and the invasive type. Elevated levels of both tumor markers meant concurrent elevation of AFP $>$ 20 ng/mL and DCP $>$ 40 AU/L).

Postoperative follow-up and definition of recurrence pattern

Patients were followed up monthly while tumor markers were measured every month, ultrasonography was performed every 3 months, and dynamic CT or MRI was performed every 6 months for the first 2 years after the operation. After 2 years, the follow-up period was determined according to the likelihood of recurrence, but the imaging schedule remained principally the same. Additional imaging studies were performed if recurrence was suspected. Dynamic CT/MRI and/or CT angiography were used to determine the presence of multiple recurrent tumors. CT, MRI, and scintigraphy were used to detect extrahepatic recurrence. The site and pattern of the initial recurrence were defined as follows: (1) solitary recurrence; (2) oligonodular (two or three tumor nodules) recurrence; (3) recurrence with four or more lesions; and (4) recurrence at an extrahepatic site regardless of concurrent intrahepatic recurrence. In this study, "advanced recurrence" was defined as recurrence with four or more lesions and/or extrahepatic recurrence. Neither recurrence with macroscopic vascular invasion nor concurrent intra- and extrahepatic recurrence were observed in our study population.

Design

Of the total of 233 patients with HCC, 54 had well-differentiated HCC, 145 had moderately differentiated HCC, and 34 had poorly differentiated HCC (P-HCC). The 199 HCC patients without PDC were grouped together as cases of non-poorly differentiated HCC (NP-HCC). A retrospective comparison of variables in the NP-HCC and P-HCC groups were performed (Table 1), as well as pattern of recurrence, second treatments after initial recurrence, and cumulative recurrence-free survival rate.

Subgroup analyses were conducted to investigate the influence of the surgical approach, including AR and resection margin width ($\geq$5 mm or $<$5 mm), on the recurrence-free survival of patients with small P-HCC.

Statistical analysis and ethical considerations

The data were analyzed with SPSS software ver. 21 (IBM SPSS, Chicago, IL, USA). All variables were categorized and their values were expressed as percentages. The χ^2 or Fisher's exact test was used to compare categorical variables between two groups, as appropriate. Cumulative overall survival and recurrence-free survival were determined using the Kaplan–Meier method. Differences between curves were assessed using the generalized Wilcoxon test. Multivariate analysis with Cox stepwise regression was used to investigate independent predictors

of prognosis. In multivariate analyses, all factors included in Table 1 and the presence of PDC were entered into the analyses. A p value of <0.05 was considered statistically significant.

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Results

Of the 233 patients, 175 were men and 58 were women. The median age at the time of hepatectomy was 61 years (range 35–79 years). The median tumor size was 16 mm (range 6–20 mm). There were two cases (0.9 %) of in-hospital mortality related to hepatectomy. A total of 132 patients (57 %) had recurrence and 79 patients died during the follow-up period. The median follow-up period for survivors was 69.0 months (range 5.1–239.6 months).

Clinicopathological characteristics of HCC ≤ 2 cm with PDC

The clinicopathological characteristics of tumors in each differentiation grade are shown in Table 1. Results showed that poor histologic differentiation was associated with an increased likelihood of elevated tumor markers, non-boundary macroscopic tumor appearance, and microscopic vascular invasion. Comparison of the NP-HCC and P-HCC groups revealed that concurrent elevation of the two tumor markers was the only significant difference between the two groups.

Recurrence patterns and second treatment according to the presence of PDC

Compared with NP-HCCs, P-HCCs were more likely to recur, extrahepatic recurrence was more common, and advanced recurrence in the early period after operation was more likely (Table 2). In cases of recurrence of P-HCC, four cases of advanced recurrence were of the disseminated type, and one case of extrahepatic recurrence had peritoneal dissemination (5/34, 15 %), on the other hand, recurrence with dissemination was seen in only eight cases of NP-HCCs (8/199, 4 %) ($p = 0.03$). The second treatments for initial recurrence are also shown in Table 2. TACE was significantly more frequent in the poorly differentiated group than in the non-poorly differentiated group. None of the patients received salvage liver transplantation for initial recurrence.

Table 2 Comparison of recurrence patterns and second treatment based on the presence of poorly differentiated component

	Non-poorly		Poorly $N = 34$	Non-poorly versus poorly
	Well $N = 54$	Moderately $N = 145$		
Overall recurrence (%)	26 (48)	80 (55)	26 (76)	*0.01*
Initial recurrence pattern (%)				
Solitary	23 (43)	52 (36)	13 (38)	1.00
Oligonodular	1 (2)	15 (10)	3 (9)	1.00
Four or more	2 (4)	13 (9)	6 (18)	*0.10*
Extrahepatic	0 (0)	0 (0)	4 (12)	*<0.01*
Initial recurrence pattern and time to recurrence (%)				
Recurrence within 1 year	4 (7)	18 (12)	8 (24)	0.06
Recurrence within 2 years	12 (22)	37 (26)	13 (38)	0.14
Advanced recurrence within 1 year[a]	0 (0)	4 (3)	8 (24)	*<0.01*
Advanced recurrence within 2 years[a]	0 (0)	10 (7)	8 (24)	*<0.01*
The second treatment after initial recurrence (%)				
Hepatectomy	5 (9)	21 (14)	5 (15)	1.00
RFA	15 (28)	27 (19)	6 (18)	0.66
TACE	5 (9)	29 (2)	12 (35)	*0.02*
Others	1 (2)	3 (2)	2 (6)	0.21

[a] Advanced recurrence was defined as recurrence with four or more lesions and/or extrahepatic recurrence

Comparison of prognosis based on the presence of PDC

The 5-year recurrence-free survival rate of all patients was 47 %. The recurrence-free survival curves for both groups are shown in Fig. 1. The 3-, 5-, and 10-year recurrence-free survival rates for NP-HCCs were 64, 50, and 32 % respectively, while those for P-HCCS were 39, 29, and 15 % respectively. The recurrence-free survival rates for P-HCC were significantly worse than those for NP-HCCs ($p < 0.01$).

Subgroup analyses were performed after stratifying each treatment approach by the presence of PDC (Fig. 2). In P-HCCs, AR resulted in a significantly lower recurrence rate compared to NAR ($p = 0.04$), although no significant difference was observed for NP-HCCs ($p = 0.73$). Additionally, in the analyses of resection margin width, a wider resection margin did not significantly reduce the recurrence rate compared with a narrower resection margin, regardless of the presence of PDC ($p = 0.32$, $p = 0.96$, respectively) (Fig. 3).

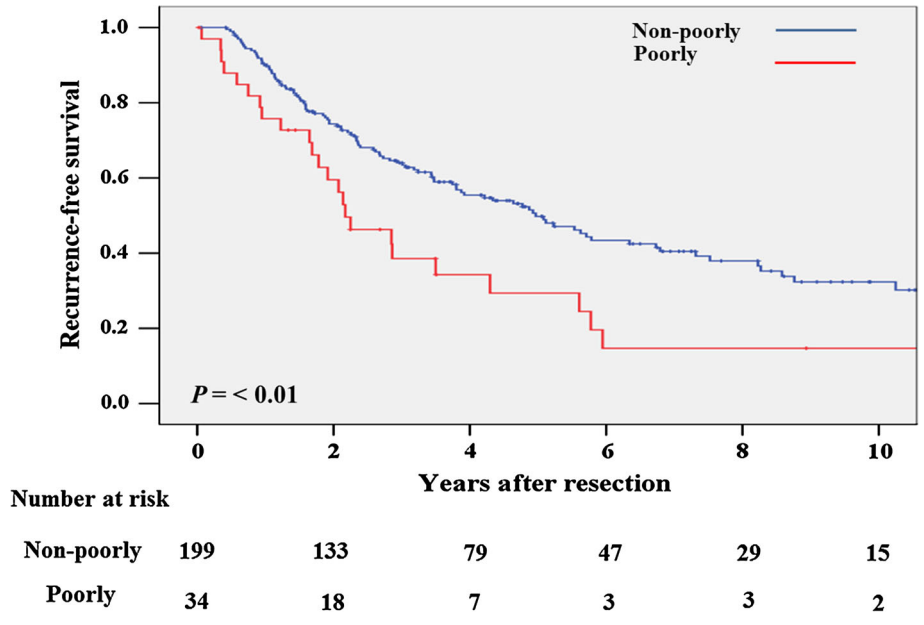

Fig. 1 The long-term prognostic impact of poorly differentiated components

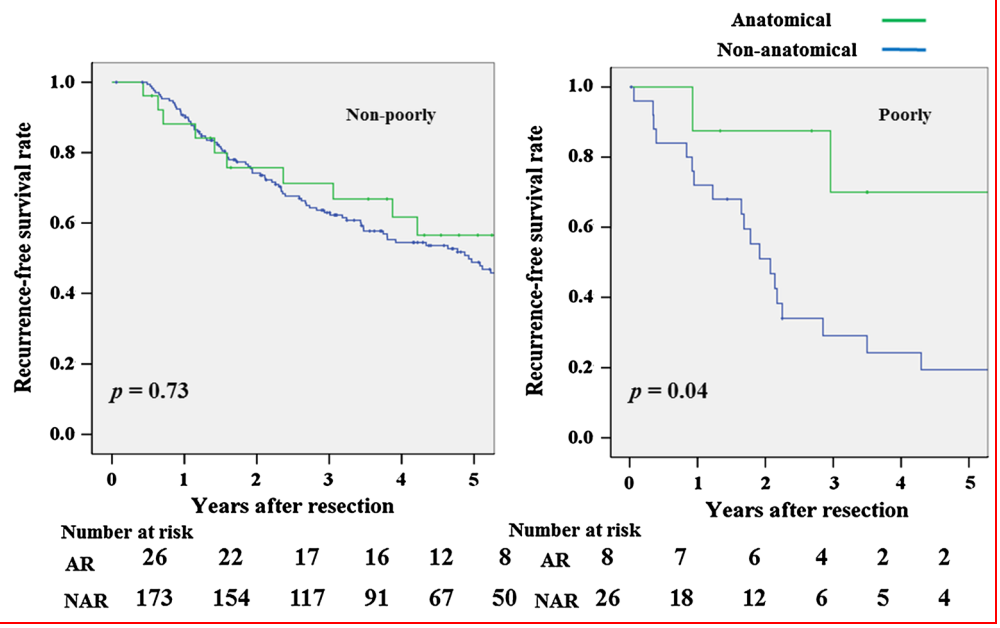

Fig. 2 The influence of surgical approach according to the presence of PDC. *PDC* poorly differentiated component *AR* anatomical resection, *NAR* non-anatomical resection

The results of univariate and multivariate analyses regarding the predictor of recurrence are shown in Table 3. In the univariate analysis, non-hepatitis B infection, hepatitis C infection, low serum platelet count, Child-Pugh grade B, high AFP level, the presence of PDC, and microscopic vascular invasion were significant predictors of recurrence. In the multivariate analysis, male sex, non-hepatitis B infection, low serum platelet count, high AFP level, and the presence of PDC were independent predictors of recurrence (Table 3). Both AR and wide resection margin were not independent predictors of recurrence.

Discussion

The existence of small HCCs with PDC has been recognized and their treatment has recently increased. However, little is known about the characteristics of these tumors because small tumors with PDC were considered to be a rare entity until recently. Additionally, these small HCC were considered as good candidates for ablation therapy and their pathological differentiation characteristics were not examined. In previous large-scale studies on hepatectomy for solitary HCC $\leq$ 2 cm, 6–23 % of patients had

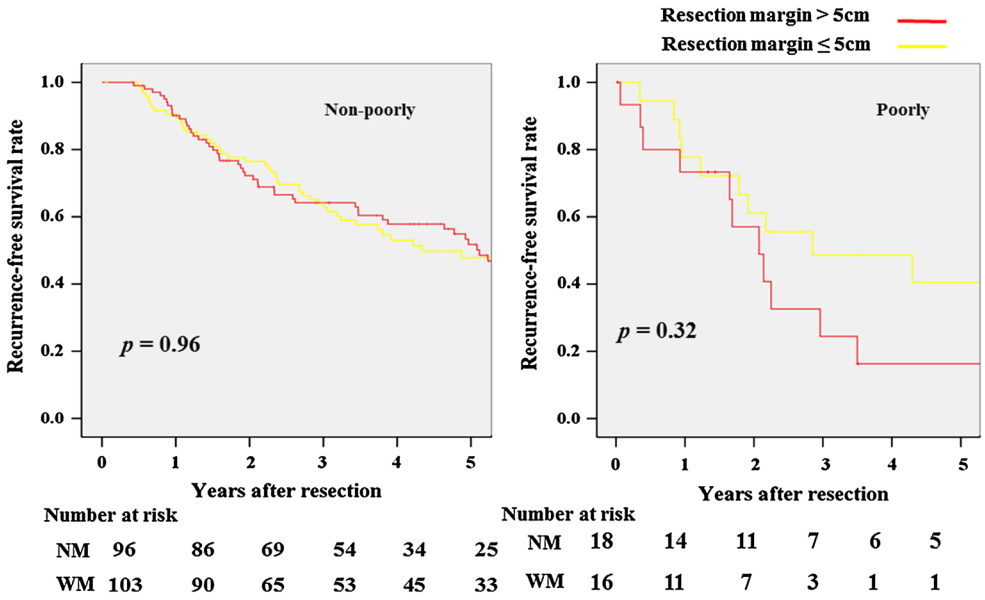

Fig. 3 The influence of the resection margin width on recurrence-free survival according to the presence of PDC. *NM* narrow margin, *WM* wide margin

PDC, which were compatible with our result [14–17]. Even if hepatectomy was the treatment of choice rather than ablation for HCCs with suspected invasive characteristics, the prevalence of small P-HCCs would not be so rare. The carcinogenesis process of small HCCs containing PDC appears to differ from the well-known multistep development theory of HCC. Further investigation into this entity from the perspective of molecular biology and genetics may enable a better understanding of the developmental process of HCC.

Current therapy guidelines do not recommend RFA for P-HCCs because of the poor outcome and the possibility of tumor seeding, moreover, P-HCCs has been considered to be a contraindication for liver transplantation in some institutions because of post-transplant recurrence and poor survival [18–20]. Therefore, the importance of preoperative detection of PDC in small HCCs has been increasingly emphasized, and recent advances in imaging modalities have made detection possible. The current study showed that elevation of both tumor markers in cases of very small solitary HCCs may be a useful preoperative predictor of the presence of PDC. Using the elevation of tumor markers to predict the presence of PDC is advantageous, especially for HCCs $\leq$ 2 cm, because imaging evaluation of small tumors remains inadequate.

The current study showed that the postoperative outcomes of P-HCCs were worse than those of NP-HCC. Univariate and multivariate analyses showed that the presence of PDC was a poor predictor of recurrence, and tumor recurrence with dissemination was observed in 15 % (5/34) of HCCs with PDC. Considering those postoperative

Table 3 The results of univariate and multivariate analysis regarding recurrence

	Univariate	Multivariate		
	P	*p*	HR	95 %CI
Male sex	0.12	<0.01	2.26	1.40–3.63
Age >60 years	0.48			
HBV infection positive	<0.01	<0.01	0.45	0.30–0.68
HCV infection positive	<0.01			
Platelet count $<10^5$ (μL)	<0.01	0.02	2.11	1.47–3.04
Child-Pugh grade B	0.04			
Liver cirrhosis	0.46			
AFP > 100 ng/mL	0.02	<0.01	1.92	1.23–3.00
DCP > 100 AU/L[b]	0.78			
Elevation of both tumor markers[b,c]	0.21			
Anatomical resection	0.34			
Surgical margin >5 mm	0.50			
The presence of PDC	<0.01	<0.01	1.99	1.25–3.16
Non-boundary macroscopic appearance)	0.23			
Capsule formation	0.28			
Microscopic vascular invasion	0.02			

HBV hepatitis B virus, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxyprothrombin

[a] Differentiation grade was determined by the differentiation component with the worst grade in the entire specimen

[b] DCP was not measured in six patients

[c] Concurrent elevation of alpha-fetoprotein (>20 ng/mL) and des-gamma-carboxyprothrombin (>40 AU/L)

outcomes, a refinement in the operative strategy for small HCCs with preoperative suspicion of PDC should be discussed. Our subgroup analyses indicated an interesting result, although the statistical validity was insufficient because the number of patients was too small and those results were speculative. The clinical impact of AR for HCC remains controversial, although various studies have shown a firm clinical benefit of AR [21–23]. Considering that a wider surgical margin did not improve postoperative recurrence, eradication of the primary tumor in addition to any micrometastases or microsatellite lesions along the portal tributaries, which are undetectable by light microscopy, may play a role in preventing recurrence caused by PDC. We speculate that performing AR regardless of the tumor size in cases with preoperatively suspected PDC might improve their poor outcomes. However, a large, prospective, randomized controlled study involving multiple institutes is necessary to determine the clinical benefit of AR in the treatment of small HCCs with PDC.

The poor outcome of hepatectomy for solitary HCCs $\leq$ 2 cm remains controversial. The results of previous three large-scale studies of hepatectomy for solitary HCC $\leq$ 2 cm are summarized in Table 4 [14–17]. One Western multicenter study showed that the presence of PDC was predictive of early recurrence but not recurrence-free survival. One study showed that the presence of microscopic vascular invasion was a predictor of recurrence but not of survival in univariate analysis, and another study showed that none of the tumor-related factors including the presence of PDC were associated with long-term survival. Although these studies did not focus on tumor differentiation grade, there was no consensus on the impact of PDC in HCC $\leq$ 2 cm. Differences in study outcomes are likely to be due to heterogeneity in etiology, liver function, and treatment selection. Another possible cause is the subjective evaluation of tumor differentiation grade, which is a limitation of our study. Currently, there is no standard, objective assessment of the presence of PDC. As a result, the histological grade is inconsistent between institutions. Several studies have demonstrated an association between tumor differentiation grade and protein or cytokine expression [24, 25]. Novel methods to assess tumor differentiation based on these studies are attractive; however, they are not yet fully established or tested in the clinical setting. Our concern about inter-institute uniformity would be resolved by establishing a simple and objective histological grading system.

Table 4 Summary of large-scale studies on hepatectomy for solitary HCC $\leq$ 2 cm

Authors	*N*	Study period	Etiology	LC (%)	P-HCC (%)	MVI (%)	SL	Main results regarding postoperative prognosis
Midorikawa et al. 2013	248	1982-2011	NA	52	6	21	NA	None of P-HCC, MVI, and SL were not predictor of survival in multivariate analysis
Yamashita et al. 2012	149	1990–2009	Hep B 13 % Hep C 80 % Others NA	55	24	29	3	MVI was a predictor of recurrence in univariate analysis No multivariate analysis was performed
Roayaie et al. 2013	132	1995–2010	Hep B 37 % Hep C 52 % Others 11 %	67	16	27	12	SL and LC were poor predictors of recurrence in multivariate analysis SL and low serum platelet were poor predictors of survival P-HCC was a predictor of recurrence within 2 years
Shindoh et al. 2013	155	1981–2011	Hep B 37 % Hep C 38 % Others NA	60	18	26	NA	MVI was not a poor predictor of survival No multivariate analysis was performed
Sasaki et al. 2013	233	1993–2012	Hep B 32 % Hep C 59 % Others 9 %	69	15	17	2	P-HCC was a poor predictor of recurrence in multivariate analysis Extrahepatic recurrence was significantly frequent in P-HCC Early advanced recurrence was significantly frequent in P-HCC 15 % of P-HCC had recurrence with dissemination even after hepatectomy

LC liver cirrhosis, *P-HCC* hepatocellular carcinoma with poorly differentiated component, *MVI* positive for microscopic vascular invasion, *SL* presence of satellite lesion, *Hep B* positive for hepatitis B surface antigen, *Hep C* positive for hepatitis C antibody, *NA* not available

The current study is the first study that examined the influence of poor histological differentiation grade in HCCs $\leq$ 2 cm, although it was a retrospective study with a relatively small sample size from a single center. A multicenter large-scale study is needed to confirm our finding. Moreover, our study did analyze the influence of liver cirrhosis because of the small number of patients. We have to analyze again after splitting the patients into cirrhotic and non-cirrhotic groups in the future to confirm our findings.

In conclusion, our result indicated that the presence of PDC should be considered in cases of small solitary HCCs $\leq$ 2 cm with an elevation of both tumor markers. Moreover, significantly worse post-hepatectomy outcomes such as early advanced recurrence or recurrence with dissemination should be taken into account if PDC is present even in HCCs $\leq$ 2 cm.

Acknowledgments The author is grateful to Dr. Daisuke Morioka and Dr. William Ng whose comments and suggestions were valuable to our study.

References

1. Kenmochi K, Sugihara S, Kojiro M (1987) Relationship of histologic grade of hepatocellular carcinoma (HCC) to tumor size, and demonstration of tumor cells of multiple different grades in single small HCC. Liver 7:18–26
2. Kudo M (2009) Multistep human hepatocarcinogenesis: correlation of imaging with pathology. J Gastroenterol 44:112–118
3. Takayama T, Makuuchi M, Kojiro M, Lauwers GY, Adams RB, Wilson SR et al (2008) Early hepatocellular carcinoma: pathology, imaging, and therapy. Ann Surg Oncol 15(4):972–978
4. Livraghi T, Lazzaroni S, Meloni F, Solbiati L (2005) Risk of tumour seeding after percutaneous radiofrequency ablation for hepatocellular carcinoma. Br J Surg 92(7):856–858
5. Imamura J, Tateishi R, Shiina S, Goto E, Sato T, Ohki T et al (2008) Neoplastic seeding after radiofrequency ablation for hepatocellular carcinoma. Am J Gastroenterol 103(12):3057–3062
6. Nakanishi M, Chuma M, Hige S, Omatsu T, Yokoo H, Nakanishi K et al (2012) Relationship between diffusion-weighted magnetic resonance imaging and histological tumor grading of hepatocellular carcinoma. Ann Surg Oncol 19(4):1302–1309
7. Kudo M, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O et al (2011) Management of hepatocellular carcinoma in Japan: consensus-based clinical practice guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. Dig Dis 29(3):339–364
8. Kawamura Y, Ikeda K, Hirakawa M, Yatsuji H, Sezaki H, Hosaka T et al (2010) New classification of dynamic computed tomography images predictive of malignant characteristics of hepatocellular carcinoma. Hepatol Res 40(10):1006–1014
9. Sasaki A, Kai S, Iwashita Y, Hirano S, Ohta M, Kitano S (2005) Microsatellite distribution and indication for locoregional therapy in small hepatocellular carcinoma. Cancer 103(2):299–306
10. Zhou XP, Quan ZW, Cong WM, Yang N, Zhang SH, Zhang SH et al (2007) Micrometastasis in surrounding liver and the minimal length of resection margin of primary liver cancer. World J Gastroenterol 13(33):4498–4503
11. Sasaki K, Matsuda M, Ohkura Y, Kawamura Y, Hashimoto M, Ikeda K et al (2013) Minimum resection margin should be based on tumor size in hepatectomy for hepatocellular carcinoma in hepatoviral infection patients. Hepatol Res 43(12):1295–1303
12. Liver Cancer Study Group of Japan (2010) The general rules for the clinical and pathological study of primary liver cancer, 3rd edn. Kanehara, Tokyo
13. Hirohashi S, Blum HE, Ishak KG (2000) Tumours of the liver and intrahepatic bile ducts. In: Hamilton SR, Aaltonen LA (eds) World Health Organisation Classification of Tumours: Pathology and genetics of tumours of the digestive system. IARC Press, Lyon, pp 157–202
14. Midorikawa Y, Takayama T, Shimada K, Nakayama H, Higaki T, Moriguchi M et al (2013) Marginal survival benefit in the treatment of early hepatocellular carcinoma. J Hepatol 58(2):306–311
15. Yamashita Y, Tsuijita E, Takeishi K, Fujiwara M, Kira S, Mori M et al (2012) Predictors for microinvasion of small hepatocellular carcinoma $\leq$2 cm. Ann Surg Oncol 19(6):2027–2034
16. Roayaie S, Obeidat K, Sposito C, Mariani L, Bhoori S, Pellegrinelli A et al (2013) Resection of hepatocellular cancer $\leq$2 cm: results from two Western centers. Hepatology 57(4):1426–1435
17. Shindoh J, Andreou A, Aloia TA, Zimmitti G, Lauwers GY, Laurent A et al (2013) Microvascular invasion does not predict long-term survival in hepatocellular carcinoma up to 2 cm: reappraisal of the staging system for solitary tumors. Ann Surg Oncol 20(4):1223–1229
18. Bruix J, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases (2005) Management of hepatocellular carcinoma. Hepatology 42(5):1208–1236
19. Tamura S, Kato T, Berho M, Misiakos EP, O'Brien C, Reddy KR et al (2001) Impact of histological grade of hepatocellular carcinoma on the outcome of liver transplantation. Arch Surg 136(1):25–30 discussion 31
20. DuBay D, Sandroussi C, Sandhu L, Cleary S, Guba M, Cattral MS et al (2011) Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. Ann Surg 253(1):166–172
21. Hasegawa K, Kokudo N, Imamura H, Matsuyama Y, Aoki T, Minagawa M et al (2005) Prognostic impact of anatomic resection for hepatocellular carcinoma. Ann Surg 242(2):252–259
22. Eguchi S, Kanematsu T, Arii S, Okazaki M, Okita K, Omata M et al (2008) Comparison of the outcomes between an anatomical subsegmentectomy and a non-anatomical minor hepatectomy for single hepatocellular carcinomas based on a Japanese nationwide survey. Surgery 143(4):469–475
23. Chen J, Huang K, Wu J, Zhu H, Shi Y, Wang Y et al (2011) Survival after anatomic resection versus nonanatomic resection for hepatocellular carcinoma: a meta-analysis. Dig Dis Sci 56(6):1626–1633
24. Harimoto N, Taguchi K, Shirabe K, Adachi E, Sakaguchi Y, Toh Y et al (2010) The significance of fibroblast growth factor receptor 2 expression in differentiation of hepatocellular carcinoma. Oncology. 78(5–6):361–368
25. Shi XH, Zheng Y, Sun Q, Cui J, Liu QH, Qü F et al (2011) DEC1 nuclear expression: a marker of differentiation grade in hepatocellular carcinoma. World J Gastroenterol 17(15):2037–2043