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RESEARCH ARTICLE

The tumor diameter cut-off for predicting microscopic intrahepatic metastasis of hepatocellular carcinoma patients without treatment history differs from that of hepatocellular carcinoma patients with a treatment history

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Abstract

Background and aim Intrahepatic metastasis (IM) of hepatocellular carcinoma (HCC) occurs via vascular invasion; the tumor diameter that affects the risk of micro intra-hepatic metastasis (MIM) should be larger than that which affects the risk of micro vessel invasion (MVI). The aim of the present study was to determine the optimum tumor diameter cut-off value for predicting the presence of MIM in HCC patients without treatment history and HCC patients with a treatment history and to compare these diameters between cases of MVI and MIM.

Methods This retrospective study included 621 patients without macroscopic vessel invasion or intrahepatic metastasis on preoperative imaging who underwent hepatectomy. The cut-off tumor diameter for predicting the presence of MIM was determined by a receiver operating characteristic curves analysis.

Results The optimum cut-off value for predicting the presence of MIM in HCC patients without treatment history was 43 mm. In contrast, the optimum cut-off value for predicting the presence of MIM in HCC patients with a treatment history was 20 mm. Among 46 HCC patients with MIM without treatment history, there were 20 patients with MIM without MVI who were considered to have potential multi-centric (MC) tumors rather than IM. The cumulative overall survival rates in patients with MIM without MVI (potential MC) was significantly better than that in patients with both MIM and MVI ($P = 0.022$).

Conclusions The tumor diameter cut-off value for predicting MIM differed between HCC patients without treatment history and with a treatment history and slightly smaller than those for predicting MVI beyond our expectation.

Keywords Hepatocellular carcinoma · Tumor diameter · Microscopic intrahepatic metastasis · Potential multi-centric metastasis · Receiver operating characteristic curve

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies and it is characterized by insidious onset at an early stage, followed by microscopic vessel invasion (MVI) and microscopic intrahepatic metastasis (MIM) with tumor growth [1–3]. It is generally believed that patients with small tumors have a more favorable prognosis than those with large tumors [2, 4–7], suggesting that tumor size is an important prognostic factor; this notion has subsequently been adopted in several staging systems [8, 9]. However, some studies have shown that tumor size itself is not a significant prognostic factor despite the correlation between tumor size and MIM [7, 10–13]. Thus, the precise impact of the tumor size on the prognosis has remained unclear.

We previously reported that the predictors of MVI differ between HCC patients without treatment history and HCC patients with a treatment history and that the tumor diameter was an independent predictive factor that should be considered when predicting MVI, especially in HCC patients with a treatment history [14]. In theory, one of the major metastatic forms of MIM depends on the presence of vascular

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invasion [15]; the diameter of the tumor that affects the risk of MIM should be larger than that which affects the risk of MVI. Based on the results of previous studies [4–7, 14, 15], we hypothesized that the tumor diameter that induces MIM would be larger than or equal to that which induces MVI and it would differ between HCC patients without treatment history and HCC patients with treatment history (similar to MVI).

The aims of the present study were to determine the optimum tumor diameter cut-off value for predicting the presence of MIM in HCC patients without treatment history and HCC patients with a treatment history and to compare these diameters between cases of MVI and MIM.

Methods

Patients and methods

A total of 697 patients underwent hepatectomy with curative intent at the Division of Hepato-Biliary-Pancreatic Surgery, Shizuoka Cancer Center Hospital, between September 2002 and June 2017. We retrospectively reviewed the database of this hospital until January 2018. This study was retrospective, and we obtained approval from the Institutional Review Board of Shizuoka Cancer Center for the exception of patients' consent "29-J11-29-1-3".

All of the patients who were included in the study had undergone computed tomography (CT) before surgery. Between 2003 and 2008, CT scans were performed with a 16-detector CT scanner (Aquilion 16; Toshiba Medical Systems, Tokyo, Japan), and after October 2008, the scans were performed with a 320-detecter CT scanner (Aquilion ONE; Toshiba Medical Systems, Tochigi, Japan). The scanning parameters were as follows: 1-mm slice thickness, reconstruction of the data at 1-mm intervals (0.5 mm overlap), rotation time 0.5 s, tube voltage 135 kV (peak), and tube current 350–400 mA. Images were obtained after the intravenous administration of 150 mL of 350 mgI/mL iopamidol (Iopamiron; Nihon Schering Co., Ltd, Tokyo, Japan) using a calibrated power injector (Auto Enhance A-50; Nemoto Kyorindo, Tokyo, Japan) at a rate of 4 mL/s. The late arterial phase was started 35 s after the injection. All of the CT images were evaluated by independent reviewer (TA) who did not have access to the original interpretations or outcomes. In the late arterial phase, the diameter of the tumor in each axial, coronal and sagittal phase was measured before surgery and the largest diameter was applied in the present study. Based on the radiologists' report, the presence of macroscopic vessel invasion and intrahepatic metastasis was also judged.

In patients with multiple tumors, the diameter of the largest tumor was applied in the present study. Subsequently, patients were classified into two groups: a HCC without treatment history group and a HCC with treatment history group. The analyses for determining the cut-off values of tumor diameter for predicting each MVI and MIM were performed after classifying patients into these two groups. All of the patients underwent preoperative viral serological testing of tumor markers such as alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP), a laboratory assessment of the liver function. The liver function was assessed using the Child–Pugh classification [16] and liver damage criteria [8], including the indocyanine green retention rate at 15 min. All of the patients presented with a confirmed diagnosis of HCC after surgical pathology. The resected specimens were cut into serial 2–3 mm-thick slices and fixed in 10% formalin to facilitate careful gross and histopathological examinations. Each of the liver slices was embedded in paraffin, cut into 4-mm sections, and stained and hematoxylin and eosin. Based on the pathological report, MVI was defined as the presence of either microscopic portal vein invasion or venous vein invasion and MIM was defined as the presence of microscopic intrahepatic metastases in the present study [8]. The differentiation between multiple tumor and MIM was done before surgery in principle: multiple tumors were defined as the tumors visible in preoperative CT, whereas MIM was defined as the tumors invisible in preoperative CT. The tumor stage was assessed based on the seventh edition of the Union Internationale Contra le Cancer classification (UICC) [9].

The surgical procedure and the extent of hepatectomy in each patient were decided in a weekly surgical conference. The details of the surgical strategy and procedure have been previously reported [17]. The types of hepatectomies were defined according to the Brisbane 2000 terminology as minor (two liver segments or less) or major (three liver segments or more) [18].

The patients were subjected to a physical examinations and blood tests every 3 months after surgery. Serial CT or liver ultrasonography was performed in each patient every three to six months. When recurrence of HCC was found, the most appropriate therapy, such as repeat hepatectomy, transcatheter arterial chemoembolization (TACE), radiofrequency ablation (RFA), or sorafenib, was applied, after considering the patient's liver function and tumor factors. For the analysis of the overall survival rate, the follow-up period ended at the time of death from HCC. The remaining patients were censored at the last follow-up visit until January 2018.

The cut-off points for the laboratory data were defined as the upper limit of normal applied at our institution, and the cut-off value for age was defined as the median value. The cut-off values for tumor diameter for predicting the presence of MVI and/or MIM were determined using receiver operating characteristic (ROC) curves and Youden's index.

Statistical analyses

Continuous variables are presented as the median and range and were compared using the Mann–Whitney *U* test. The categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. The cumulative relapse-free and overall survival curves were analyzed using the Kaplan–Meier method and compared using the log-rank test. A Cox proportional hazards model was used for the univariate and multivariate analyses, and all factors found to be significant predictors of the relapse-free and overall survival ($P < 0.10$) in the univariate analysis were entered into the multivariate analysis. The multivariate analysis was performed using a backward stepwise selection model. All statistical analyses were performed using the SPSS 24.0 software package (SPSS, Inc., Chicago, IL, USA), and *P* values of ≤ 0.05 in two-tailed tests were considered to be significant.

Results

Patient characteristics

Among the 697 patients, 74 and 2 patients were excluded from this analysis due to the presence of macroscopic vessel invasion and intrahepatic metastases on preoperative imaging and a lack of pathological results, respectively. The remaining 621 patients with HCC were ultimately included for an evaluation in this study. The patient characteristics are shown in Table 1. There were 458 and 163 patients in HCC without treatment history and HCC with treatment history, respectively. Among the 163 HCC patients with treatment history, 71, 34, 49, and 9 patients underwent surgical resection, RFA, TACE, and other procedures as the most recent treatment before surgery. The median tumor diameter was 31 mm (range, 3–180 mm). MVI and MIM were identified in 112 (18.0%) and 63 (10.1%) patients, respectively.

Table 1 Clinicopathological characteristics of the patients

Characteristics	
Age (years)[a]	70 (38–87)
Gender (male/female)	495/126
Etiology of liver disease (viral/non-viral)	397/224
HBsAg-positive (%)	116 (18.7)
Anti-HCV Ab-positive (%)	284 (45.7)
Treatment history of HCC (present)	163 (26.2)
Surgical resection	71
RFA	34
TACE	49
The other	9
Major resection (present)	148 (23.8)
Anatomical resection (present)	321 (51.7)
Albumin (g/L)[a]	41 (23–51)
Total serum bilirubin (mg/dL)[a]	0.6 (0.1–2.3)
AST (IU/L)[a]	35 (15–169)
ALT (IU/L)[a]	33 (11–281)
Platelet count ($\times 10^4$/μl)[a]	15.2 (4.8–79.0)
PT (%)[a]	87 (53–130)
AFP (ng/mL)[a]	10.8 (0.8–214,812)
DCP (mAU/mL)[a]	111 (1–446,000)
Child–Pugh classification (A/B)	610/11/0
Cirrhosis (present)	181 (29.1)
Maximum tumor diameter (mm)[a]	31 (3–180)
Tumor number (multiple)	160 (25.7)
Microscopic vessel invasion (present)[b]	112 (18.0)
Microscopic intrahepatic metastases (present)[b]	63.1 (10.1)
Tumor stage (I/II/III)	389/180/52

HBsAg hepatitis B surface antigen, *HCV* hepatitis C virus, *Ab* antibody, *HCC* hepatocellular carcinoma, *RFA* radiofrequency ablation, *TACE* transcatheter arterial chemoembolization, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin

Values in parentheses are percentages unless indicated otherwise; value is [a]median (range)

[b]Microscopic intrahepatic metastases and vessel invasion were diagnosed in pathological examination

Determination of the optimum cut-off value of tumor diameter for predicting the presence of MVI and MIM

ROC curves and Youden's index were calculated to determine the optimum cut-off values of tumor diameter for predicting the presence of MVI and MIM in patients with HCC without treatment history. A tumor diameter of 48 mm could predict the presence of MVI with a sensitivity of 61.3% and a specificity of 70.6% and an area under the curve (AUC) of 0.701 (Fig. 1a). Regarding the presence of MIM, a tumor diameter of 43 mm could predict it with a sensitivity of 63.8% and a specificity of 65.6%, and an AUC of 0.740 (Fig. 1b). In the HCC patients with treatment history, ROC curves showed that a tumor diameter of 24 mm could predict the presence of MVI with a sensitivity of 53.1% and a specificity of 65.6%, and an AUC of 0.632 (Fig. 1c) and showed that a tumor diameter of 20 mm could predict the presence of MIM with a sensitivity of 82.4% and a specificity of 50.0%, and an AUC of 0.664 (Fig. 1d). These cut-off values were used in the subsequent analyses.

A comparison of the clinicopathological factors between HCC patients without treatment history with and without MIM

The rate of MVI in the patients with MIM was significantly higher than in the patients without MIM (56.5% vs. 13.1%,

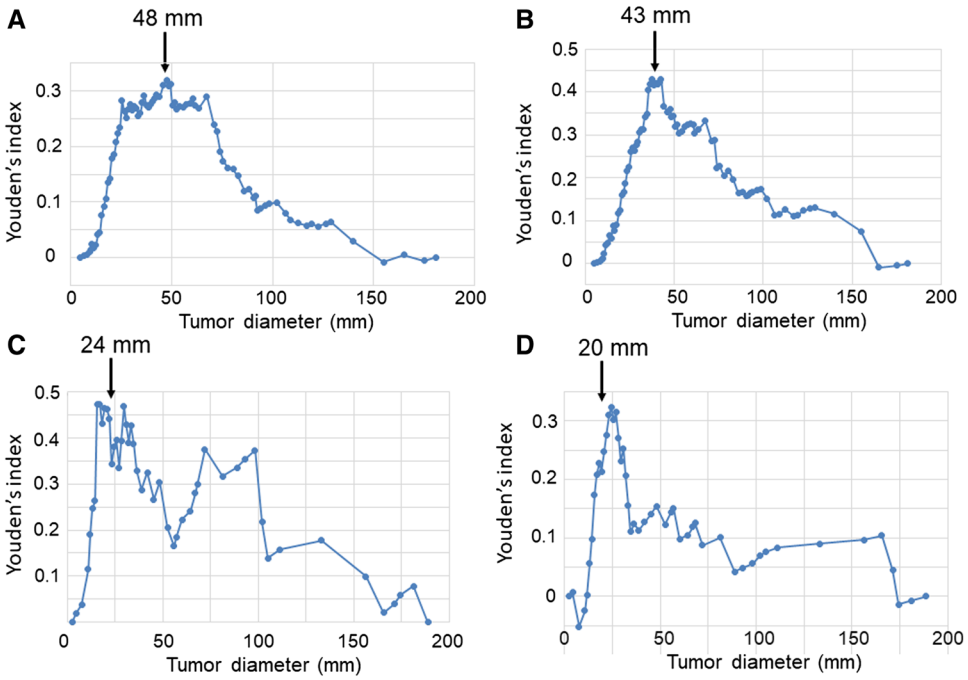

Fig. 1 Receiver operating characteristic curves and Youden's index for the tumor diameter for predicting the presence of MVI and MIM in HCC patients without treatment history and with a treatment history (arrows show the each optimum cut-off point of tumor diameter). **a** MVI in HCC patients without treatment history. **b** MIM in HCC patients without treatment history. **c** MVI in HCC patients with a treatment history. **d** MIM in HCC patients with a treatment history

$P < 0.001$), but there were 20 patients with MIM without MVI who were considered to have potential multi-centric (MC) tumors rather than intrahepatic metastasis (IM) (Table 2). The cumulative overall and relapse-free survival rates in patients with MIM were significantly poorer than in patients without MIM (Fig. 2a, b, both $P < 0.001$). Moreover, the cumulative overall survival rates in patients with MIM without MVI (potential MC) was significantly better than in patients with both MIM and MVI (Fig. 3, $P = 0.022$).

Univariate and multivariate analyses to identify the predictors of MIM in HCC patients without treatment history

Four preoperative factors were identified as the candidate predictors of the presence of MIM. After converting the continuous variables to categorical variables, an ROC curve analysis was performed to determine the cut-off values for the AST level (35 IU/L), DCP (130 mAU/mL) levels, and the tumor diameter (43 mm) (Fig. 1b). The odds ratios (ORs) for possible determinants of the presence of MIM, which were determined in the univariate logistic regression analyses, are shown in Table 3. In the multivariate analysis, the following factors remained as significant independent predictors of MIM in the HCC patients without treatment history: tumor diameter > 43 mm (OR 6.49, 95% confidence interval [CI] 3.11–13.5, $P < 0.001$) and AST > 35 IU/L (OR 2.58, 95% CI 1.29–5.15, $P = 0.007$) (Table 3).

Moreover, the cumulative overall and relapse-free survival rates in patients with tumor diameter > 43 mm were significantly poorer than in patients with tumor diameter ≤ 43 mm (Fig. 4a, b, $P = 0.025$ and $P = 0.005$, respectively).

Univariate and multivariate analyses of the prognostic factors for the overall survival in HCC patients without treatment history

In the multivariate analysis, the presence of MIM (hazard ratio [HR] 2.91, 95% CI 1.85–4.59, $P = 0.001$), age ≥ 70 years (HR 1.78, 95% CI 1.28–2.49, $P = 0.001$), Albumin < 40 g/dL (HR 1.61, 95% CI 1.15–2.25, $P = 0.005$), DCP ≥ 40 mAU/mL (HR 1.56, 95% CI 1.06–2.29, $P = 0.025$), and AFP ≥ 20 ng/mL (HR 1.47, 95% CI 1.06–2.04, $P = 0.022$) remained significant independent predictors of the overall survival (Table 4).

A comparison of the clinicopathological factors between HCC patients with a treatment history with and without MIM

The median tumor diameter in the patients with MIM was significantly larger than in the patients without MIM ($P = 0.027$). However, there were no correlations between the presence of MVI and MIM ($P = 0.106$) in HCC patients with a treatment history (Table 5). The cumulative overall and relapse-free survival rates in patients with MIM were significantly poorer than in patients without MIM (Fig. 2c, d, $P < 0.001$ and $P = 0.049$, respectively). There were no significant differences of the cumulative overall and relapse-free survival rates between the patients with MIM without

Table 2 Comparisons of clinicopathological factors between HCC patients without treatment history with and without microscopic intrahepatic metastasis (MIM)

	With MIM $n=46$	Without MIM $n=412$	P
Age (years)[a]	71 (45–80)	70 (39–87)	0.498
Gender (male/female)	38/8	326/86	0.702
Etiology of liver disease (viral/non-viral)	20/26	252/160	0.136
Major resection (present)	21 (45.7)	95 (23.1)	0.002
Anatomical resection (present)	33 (71.7)	231 (56.1)	0.042
Albumin (g/L)[a]	40 (29–46)	41 (23–51)	0.080
Total serum bilirubin (mg/dL)[a]	0.6 (0.4–1.8)	0.6 (0.2–2.3)	0.662
AST (IU/L)[a]	41 (22–90)	35 (15–143)	0.007
ALT (IU/L)[a]	36 (5–94)	35 (11–281)	0.442
Platelet count ($\times 10^4$/μl)[a]	18.5 (7.8–34.9)	15.4 (4.8–41.6)	0.089
PT (%)[a]	86 (53–114)	88 (55–130)	0.195
AFP (ng/mL)[a]	24.2 (1.4–199,133)	10.7 (1.2–214,812)	0.147
DCP (mAU/mL)[a]	964 (18–345,000)	124 (1–446,000)	< 0.001
Child–Pugh (B)	0 (0)	9 (2.2)	1.000
Cirrhosis (present)	8 (17.4)	113 (27.4)	0.305
Tumor diameter (mm)[a]	70 (14–160)	35 (6–180)	< 0.001
Tumor number (multiple)	12 (26.1)	96 (23.3)	0.714
Tumor differentiation (well/moderately/poorly)	2/41/3	74/324/13	0.038
Microscopic vessel invasion (present)[b]	26 (56.5)	54 (13.1)	< 0.001
Tumor stage (I/II/III)	18/22/6	280/106/26	0.001

HCC hepatocellular carcinoma, *MIM* microscopic intra-hepatic metastasis, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin

Values in parentheses are percentages unless indicated otherwise; [a]value is expressed as the median (range)

[b]Microscopic intrahepatic metastasis and vessel invasion were diagnosed in pathological examination

MVI (potential MC) and the patients with both MIM and MVI ($P=0.226$ and $P=0.532$, respectively).

Univariate and multivariate analyses to identify the predictors of MIM in HCC patients with a treatment history

Two preoperative factors were identified as the candidate predictors of the presence of MIM. After converting the continuous variables to categorical variables, an ROC curve analysis was performed to determine the cut-off values for the DCP level (40 mAU/mL) and the tumor diameter (20 mm). The odds ratios (ORs) for possible determinants of the presence of MIM, which were determined in the univariate logistic regression analyses, are shown in Table 6. In the multivariate analysis, DCP > 40 mAU/mL (OR 5.77, 95% CI 1.25–26.7, $P=0.025$) remained as only significant independent predictor of MIM in the HCC patients with treatment history (Table 6). The cumulative overall survival rate in patients with tumor diameter > 20 mm was significantly poorer than in patients with tumor diameter ≤ 20 mm (Fig. 4c, $P=0.001$). In contrast, there was no significant difference of relapse-free survival between the patients with tumor diameter > 20 mm and patienct with tumor diameter ≤ 20 mm (Fig. 4d, $P=0.449$).

Univariate and multivariate analyses of the prognostic factors for overall survival in HCC patients with treatment history

In the multivariate analysis, the presence of MIM (HR 3.09, 95% CI 1.53–6.25, $P=0.002$), the presence of MVI (HR 2.44, 95% CI 1.34–4.46, $P=0.004$), and a tumor diameter ≥ 24 mm (HR 2.22, 95% CI 1.32–3.74, $P=0.003$) remained significant independent predictors of the overall survival (Table 7).

Discussion

The present study showed that the tumor diameter cut-off value for predicting the presence of MIM was slightly smaller that predicting MVI in both HCC patients without treatment history and with a treatment history. Moreover, the cumulative overall survival rates in patients with MIM without MVI (potential MC) was significantly better than that in patients with both MIM and MVI. The presence of

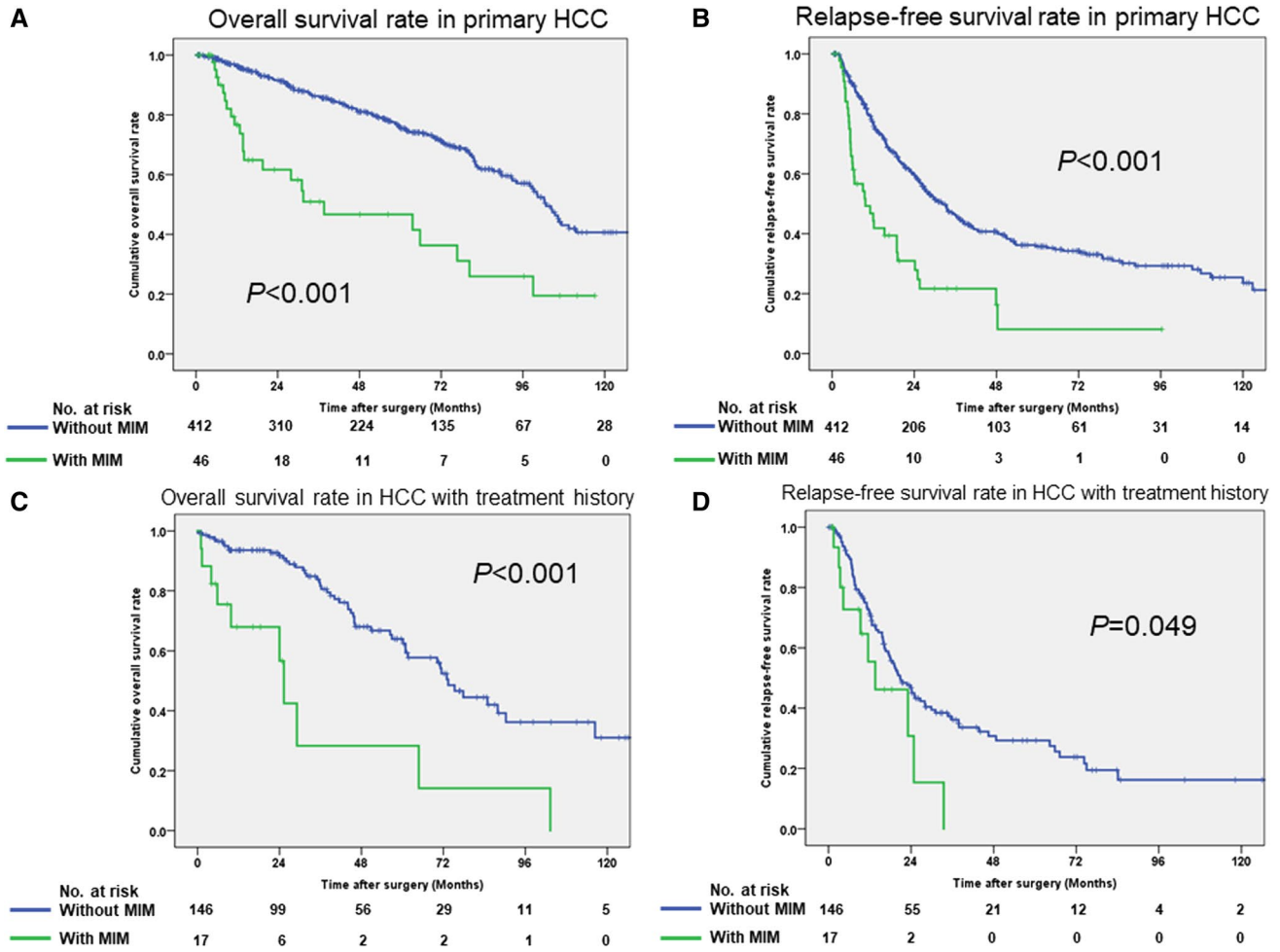

Fig. 2 Survival curves of patients who underwent hepatectomy using the Kaplan–Meier method. **a** Overall survival curve classified by the presence of MIM in HCC patients without treatment history. **b** Relapse-free survival curve classified by the presence of MIM in HCC patients without treatment history. **c** Overall survival curve classified by the presence of MIM in HCC patients with a treatment history. **d** Relapse-free survival curve classified by the presence of MIM in HCC patients with a treatment history

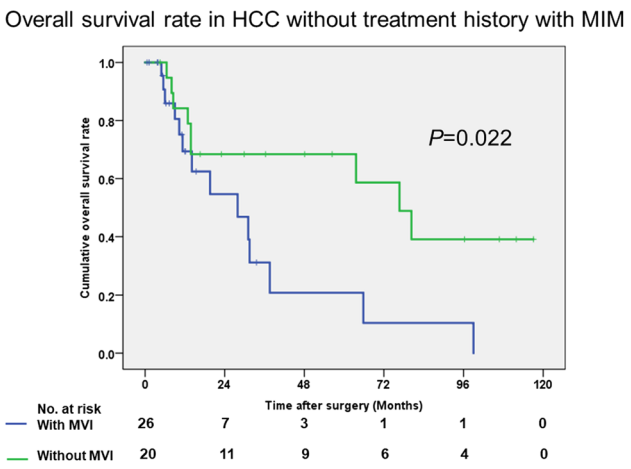

Fig. 3 Overall survival curve classified by the presence of MVI in HCC patients without treatment history with MIM

MIM was significant prognostic factors for overall survival in both the HCC patients without treatment history and with a treatment history.

This study contains several important results. First, although the tumor diameter cut-off value for predicting MVI and MIM was almost same, the tumor diameter cut-off value for predicting MIM was unexpectedly slightly smaller than that of MVI. This trend was confirmed for HCC patients without treatment history and with a treatment history. However, these results seemed to contradict the hypothesis that MIM, which is one of the major metastatic forms of HCC, occurs after vascular invasion [15]. Moreover, the lack of any correlation between the presence of MVI and MIM in HCC patients with a treatment history was a surprising result.

Among HCC patients without treatment history, we identified 20 patients with MIM without MVI. Thus, the present

Table 3 The predictor of MIM in the patients with HCC without treatment history

Variables	Univariate		Multivariate	
	Odds ratio (95% confidence interval)	*P*	Odds ratio (95% confidence interval)	*P*
Major resection (present)	2.80 (1.50–5.23)	0.001		
Anatomical resection (present)	1.99 (1.02–3.89)	0.044		
AST (> 35 IU/L)	2.64 (1.35–5.16)	0.005	2.58 (1.29–5.15)	0.007
DCP (> 130 mAL/mL)	3.73 (1.80–7.71)	< 0.001		
Tumor diameter (> 43 mm)	6.63 (3.20–13.7)	< 0.001	6.49 (3.11–13.5)	< 0.001
Tumor differentiation (well)	1			
Moderately	4.68 (1.11–19.7)	0.036		
Poorly	8.54 (1.30–56.2)	0.026		

MIM microscopic intra-hepatic metastasis, *HCC* hepatocellular carcinoma, *AST* aspartate aminotransferase, *DCP* des-gamma-carboxy prothrombin

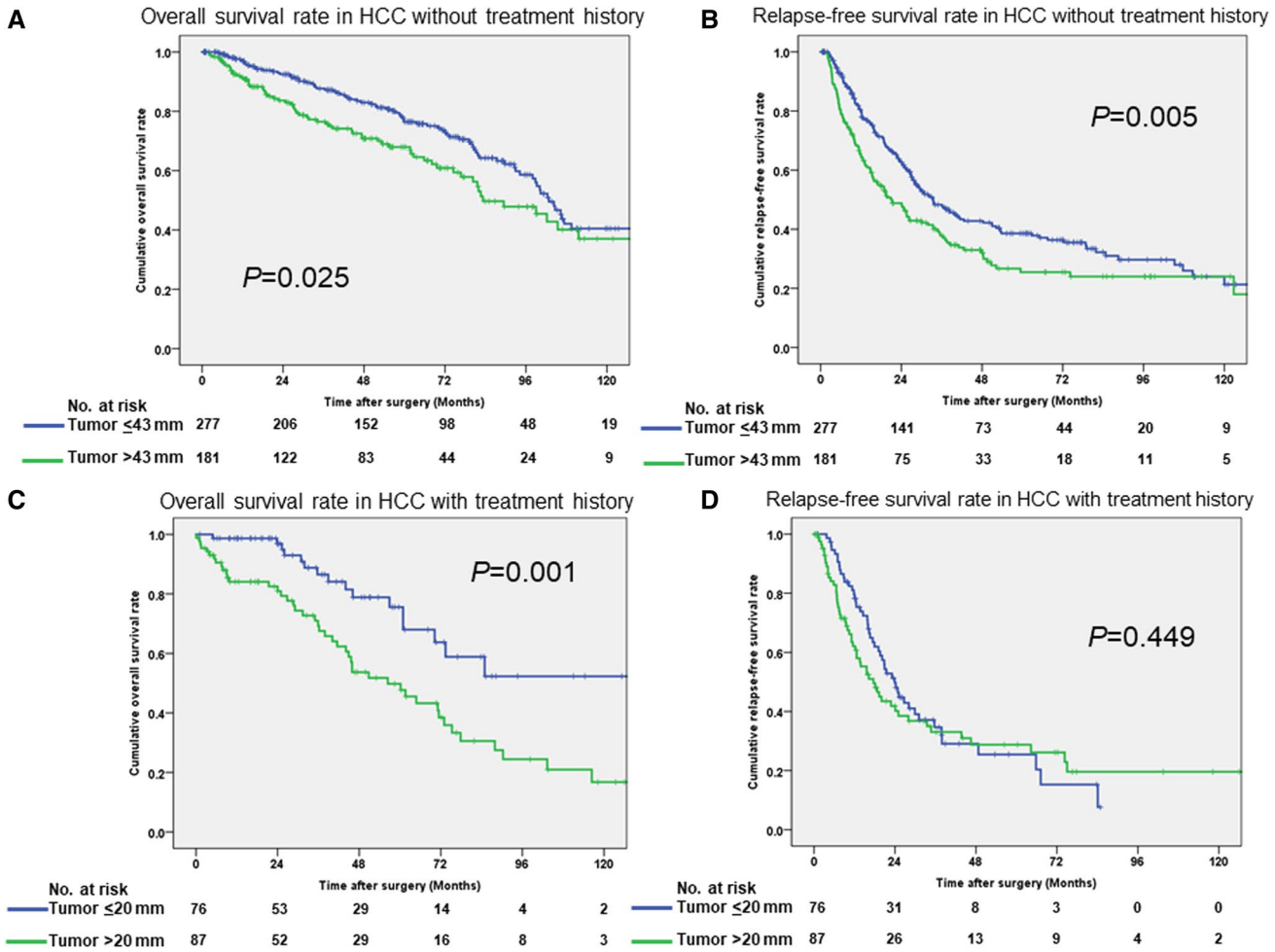

Fig. 4 Survival curves of patients who underwent hepatectomy using the Kaplan–Meier method. **a** Overall survival curve classified by the tumor diameter > 43 mm in HCC patients without treatment history. **b** Relapse-free survival curve classified by the tumor diameter > 43 mm in HCC patients without treatment history. **c** Overall survival curve classified by the tumor diameter > 20 mm in HCC patients with a treatment history. **d** Relapse-free survival curve classified by the tumor diameter > 20 mm in HCC patients with a treatment history

Table 4 Prognostic factors for the overall survival in HCC patients without treatment history by univariate and multivariate analyses

Variables	Univariate		Multivariate	
	Hazard ratio (95% confidence interval)	*P*	Hazard ratio (95% confidence interval)	*P*
Age (≥ 70 years)	1.69 (1.21–2.35)	0.002	1.78 (1.28–2.49)	0.001
Gender (male)	1.08 (0.71–1.62)	0.726		
Etiology of liver disease (viral)	1.03 (0.74–1.43)	0.872		
Albumin (< 40 g/L)	1.52 (1.09–2.11)	0.013	1.61 (1.15–2.25)	0.005
AFP (≥ 20 ng/mL)	1.55 (1.13–2.14)	0.007	1.47 (1.06–2.04)	0.022
DCP (≥ 40 mAL/mL)	1.70 (1.16–2.49)	0.006	1.56 (1.06–2.29)	0.025
Cirrhosis (present)	1.30 (0.92–1.83)	0.139		
Child–Pugh grade (B)	1.31 (0.58–2.98)	0.514		
Major resection (present)	1.02 (0.71–1.48)	0.908		
Anatomical resection (present)	0.93 (0.68–1.29)	0.681		
Tumor diameter	1.01 (1.00–1.01)	0.004		
Tumor number (multiple)	1.38 (0.97–1.97)	0.075		
Tumor diameter (≥ 43 mm)	1.45 (1.05–2.00)	0.026		
Tumor diameter (≥ 48 mm)	1.45 (1.04–2.01)	0.028		
Microscopic vessel invasion (present)[a]	1.73 (1.17–2.55)	0.006		
Microscopic intrahepatic metastases (present)[a]	3.06 (1.96–4.79)	< 0.001	2.91 (1.85–4.59)	< 0.001

HCC hepatocellular carcinoma, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin

[a]Microscopic intrahepatic metastases and vessel invasion were diagnosed in pathological examination

Table 5 Comparisons of clinicopathological factors between HCC patients with treatment history with and without MIM

	With MIM $n = 17$	Without MIM $n = 146$	*P*
Age (years)[a]	70 (61–79)	71 (38–84)	0.824
Gender (male/female)	13/4	118/28	0.747
Etiology of liver disease (viral/non-viral)	5/12	33/113	0.549
Major resection (present)	7 (41.2)	25 (17.8)	0.046
Anatomical resection (present)	12 (70.6)	48 (32.9)	0.003
Albumin (g/L)[a]	41 (29–47)	41 (27–49)	0.498
Total serum bilirubin (mg/dL)[a]	0.6 (0.1–1.2)	0.6 (0.2–1.6)	0.254
AST (IU/L)[a]	38 (22–152)	31 (16–169)	0.103
ALT (IU/L)[a]	29 (18–117)	29 (9–191)	0.370
Platelet count ($\times 10^4$/μl)[a]	14.7 (7.6–36.0)	14.3 (5.7–79.0)	0.937
PT (%)[a]	94 (69–108)	87 (55–117)	0.494
AFP (ng/mL)[a]	11.8 (3.2–2,674)	8.9 (0.8–48,862)	0.094
DCP (mAU/mL)[a]	191 (18–89,200)	45 (1–91,200)	0.007
Child–Pugh (B)	0 (0)	2 (1.4)	1.000
Cirrhosis (present)	6 (35.3)	56 (38.4)	1.000
Tumor diameter (mm)[a]	26 (4–139)	20 (3–150)	0.027
Tumor differentiation (well/moderately/poorly)	2/14/1	31/104/11	0.609
Tumor number (multiple)	7 (41.2)	45 (30.8)	0.416
Microscopic vessel invasion (present)[b]	6 (35.3)	26 (17.8)	0.106
Tumor stage (I/II/III)	0/15/2	85/43/18	0.188

MIM microscopic intra-hepatic metastasis, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin

Values in parentheses are percentages unless indicated otherwise; [a]value is expressed as the median (range)

[b]Microscopic intrahepatic metastasis and vessel invasion were diagnosed in pathological examination

Table 6 The predictor of MIM in the patients with HCC patients with treatment history

Variables	Univariate		Multivariate	
	Odds ratio (95% confidence interval)	*P*	Odds ratio (95% confidence interval)	*P*
DCP (> 40 mAL/mL)	7.80 (1.61–33.1)	0.010	5.77 (1.25–26.7)	0.025
Tumor diameter (> 20 mm)	3.54 (1.19–10.6)	0.024		

MIM microscopic intra-hepatic metastasis, *HCC* hepatocellular carcinoma, *DCP* des-gamma-carboxy prothrombin

Table 7 Prognostic factors for the overall survival in HCC patients with treatment history by univariate and multivariate analyses

Variables	Univariate		Multivariate	
	Hazard ratio (95% confidence interval)	*P*	Hazard ratio (95% confidence interval)	*P*
Age (≥ 70 years)	1.29 (0.77–2.14)	0.332		
Gender (male)	1.71 (0.74–3.98)	0.212		
Etiology of liver disease (viral)	1.06 (0.58–1.92)	0.858		
Treatment history of HCC (surgical resection)	0.67 (0.40–1.12)	0.131		
Albumin (< 40 g/L)	1.54 (0.93–2.56)	0.097		
AFP (≥ 20 ng/mL)	1.58 (0.95–2.62)	0.079		
DCP (≥ 40 mAL/mL)	1.14 (0.67–1.93)	0.626		
Cirrhosis (present)	1.46 (0.86–2.46)	0.163		
Major resection (present)	1.44 (0.83–2.50)	0.199		
Anatomical resection (present)	1.06 (0.63–1.78)	0.821		
Tumor diameter	1.01 (1.00–1.02)	0.024		
Tumor diameter (≥ 20 mm)	2.55 (1.44–4.51)	0.001		
Tumor diameter (≥ 24 mm)	2.65 (1.59–4.42)	< 0.001	2.22 (1.32–3.74)	0.003
Tumor number (multiple)	1.51 (0.91–2.51)	0.110		
Microscopic vessel invasion (present)[a]	2.94 (1.63–5.29)	< 0.001	2.44 (1.34–4.46)	0.004
Microscopic intrahepatic metastases (present)[a]	3.96 (1.98–7.90)	< 0.001	3.09 (1.53–6.25)	0.002

HCC hepatocellular carcinoma, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin

[a]Microscopic intrahepatic metastases and vessel invasion were diagnosed in pathological examination

study suggested that the presence of either MVI or MIM does not necessarily imply the presence of the other. These findings suggested that there were potential MC lesions that were diagnosed as IM based on a pathological examination. In fact, the cumulative overall survival rate of HCC patients without treatment history with MIM without MVI (potential MC) was significantly better than that of patients with both MIM and MVI. On the other hand, it is possible to fail to notice the minute findings of MVI in the patients with MIM without MVI (potential MC). A review that showed the frequency of MVI and MIM reported that the frequency of MIM was greater than that of MVI in some studies [3].

Our previous study has already shown that the tumor diameter cut-off value for predicting MVI differs between HCC patients without treatment history and with a treatment history [14]. Furthermore, the present study showed that the cut-off tumor diameter for predicting MIM in HCC patients without treatment history was almost the double diameter that predicted MIM in HCC patients with a treatment history (43 mm vs. 20 mm). Zhong et al. [3] reported that the frequency of MIM (66.5%) in the patients with a tumor diameter of > 5 cm was markedly higher than in patients with a tumor diameter of ≤ 5 cm (MIM, 18.9% ± 11.7%). Their results were almost consistent with our own, and were relatively close to the tumor diameter cut-off value for predicting the presence of MIM in HCC patients without treatment history [3].

Many studies have also described the frequency of MIM after repeat hepatectomy or salvage hepatectomy following RFA [19–23]. The differences in the cut-off values between HCC patients without treatment history and with a treatment history suggest that MIM is likely to occur in HCC patients with a treatment history, even if their tumors are still small, as previous studies have reported [24–27]. Wu et al. revealed that the median tumor diameter decreased significantly with an increasing number of hepatectomies,

whereas the frequency of MIM was not affected by the number of hepatectomies [19]. Studies on patients undergoing ≥ 3 repeat hepatectomies in Japan showed that the median tumor diameter was < 2 cm [20, 21], which corresponds to T1 stage according to the Liver Cancer Study Group of Japan General rules for the clinical and pathological study of primary liver cancer [8]. Given the present and previous findings, a favorable prognosis might be achieved in HCC patients with a treatment history who have a tumor diameter of ≤ 24 mm, as long as a wide surgical margin can be maintained, even when not performing anatomical resection to prevent IM recurrence. However, in addition to the tumor size, the operator should consider other factors, such as tumor marker levels [28], when deciding the operative approach, as the present study showed that MVI and MIM were identified in 15.4% and 9.6% of patients with a tumor diameter of ≤ 24 mm, respectively.

The tumor diameter was not found to be a significant prognostic factor in HCC patients without treatment history from the present study, whose result was consistent with that of the paper of Lim et al. [13], in which they concluded that tumor size was not an independent prognostic factor. In contrast, it was strongly associated with the presence of MIM in HCC patients without treatment history, whose results were consistent with those of previous papers [7, 8, 10–13]. On the other hand, a tumor diameter of 24 mm was an independent prognostic factor, along with the presence of MVI and/or MIM, in HCC patients with a treatment history.

The present study included several limitations. First, several cases of MC recurrence were regarded as IM recurrence in the present study. The differentiation between these two manifestations is very important. However, the results of the present study were useful for predicting invisible tumors before treatment based on the tumor diameter, despite the fact that we were not able to accurately differentiate between MC and IM.

Second, the rate of MIM in the present study (10.0%) was relatively low in comparison to other studies [3]. However, the prevalence of MIM in the review article varied widely (4.8–66.7%) [3]. Patients with macroscopic intrahepatic metastases were initially excluded from the present study. If these patients had been included, the rate of MVI would have increased to 14.2%.

Third, the AUCs of predicting MIM in the HCC patients without treatment history and with a treatment history were comparatively low around 0.7. Those values were not ideal, while the tumor diameter determined using ROC curve was one of the significant independent predictors of MIM in the HCC patients without treatment history.

Fourth, this study was retrospective in nature and was conducted at a single center; thus, we cannot exclude the presence of a selection bias among the patients. Finally, the cut-off tumor diameter was not calculated according to the type of treatment that was most recently applied, despite the fact that the tumor behavior of recurrent HCC differs according to the most recently applied treatment. Further prospective multi-institutional studies are, therefore, needed to validate the results of the present study objectively. However, the results of the present study, which was conducted in a relatively large population (> 600 patients) with a long median duration (42.2 months), were reliable.

In conclusion, the tumor diameter cut-off for predicting MIM of HCC patients without treatment history differs from that of HCC patients with a treatment history and was slightly smaller than that of MVI. This trend was confirmed in both HCC patients without treatment history and with a treatment history. Moreover, the cumulative overall survival rate of HCC patients with MIM without MVI (potential MC) was significantly better than that of patients with both MIM and MVI.

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Compliance with ethical standards

Conflict of interest All authors have no conflict of interest regarding the current study.

Ethical approval This study confirmed to the ethical guidelines of the Declaration of Helsinki (2013 revision) and was retrospective in nature, and we obtained approval from the Institutional Review Board of Shizuoka Cancer Center for the exception of patient consent (number: 29-J11-29–1-3).

Informed consent This study was retrospective, and we obtained approval from the Institutional Review Board of Shizuoka Cancer Center for the exception of patients' consent "29-J11-29-1-3". This study was conducted in accordance with the ethical standards of the Declaration of Helsinki.

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