Multicentric Occurrence and Spread of Hepatocellular Carcinoma in Whole Explanted End-Stage Liver

Running title: Multicentric spread of HCC in Cirrhotic Liver.

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Abstract

Background. Hepatocellular carcinoma (HCC) arising from the end stages of liver cirrhosis is a fair indication for liver transplantation (LT). To pathologically investigate the multicentric occurrence of relatively early-staged HCC in cirrhosis, we studied whole explanted livers. ***Methods.*** Fourteen explanted livers from patients undergoing living donor LT (LDLT) were examined. The stage of the HCCs was judged to be within the Milan criteria (M-C; a single HCC less than 5 cm or 3 HCCs less than 3 cm). Histological examination was performed using serially sectioned specimens 5-7 millimeters in width. Characterization of preoperatively detectable and undetectable lesions was also performed. ***Results.*** In 9 patients (64.3%), a total of 34 nodules were found after whole liver histological examination (WLHE). In 5 patients (31%), the results exceeded the M-C. The characteristics of undetectable HCCs included a minute (median size 6 mm), well-differentiated appearance (80%), with indistinct margins (85.3%), and without vascular invasion (94%). There was no recurrence in any patients at the time of follow up (median follow-up period, 30.1 months). ***Conclusions.*** A

multicentric occurrence of HCCs was demonstrated in cirrhotic livers with HCCs within the M-C. Undetectable HCCs in cirrhotic livers may have no impact on recurrence after LT.

Keywords: Hepatocellular carcinoma, Liver transplantation, Whole explanted liver, Milan criteria.

Abbreviations: HCC, hepatocellular carcinoma; LT, liver transplantation; LDLT, living donor liver transplantation; MDCT, multidetector computed tomography; MRI, magnetic resonance imaging

Introduction

Patients with hepatocellular carcinoma (HCC) are a therapeutic challenge, since most tend to have chronic hepatitis or cirrhosis, which often develops into multicentric HCCs (1, 2). Liver transplantation (LT) is indicated as the treatment of choice in selected HCC patients (3-6). In 1996, Mazzaferro and his colleagues proposed criteria for indications of LT for HCC, referred to as the Milan criteria (M-C) (7). The M-C consists of the following: solitary nodules <5 cm in size or 3 nodules <3 cm for multinodular HCC, no distant metastasis, and no evidence of vascular involvement. These factors are determined by preoperative hepatic imaging modalities. In order to investigate the real spread of HCC, whole liver examination is warranted. However, most of the previously reported whole liver examinations were performed using livers obtained through autopsy (e.g., (8)).Moreover, in other studies in which incidental HCC was detected on the explanted liver, histological examination was performed only for those nodules deemed suspicious by macroscopic examination (9-16). Thus there has not been sufficient investigation of HCCs in the whole explanted liver.

In the present study, we used whole liver histological

examination (WLHE) of transplantation explants. Clinically, these livers contained relatively early stage HCC within the M-C. Therefore, precise existence of HCCs in a cirrhotic liver with early staged HCC could be determined. The detectability and characterization of preoperatively undetectable HCCs was also examined.

Patients and Methods

Patients. Between August 1997 and December 2006, 62 LDLTs were performed at our center. In the early years, we performed LDLT mostly in patients with biliary atresia with parental donors. Beginning in November 2000, however, we performed LDLT on 21 patients with cirrhotic livers who showed signs of HCC within the M-C based on multidetector computed tomography scanning (MDCT) and magnetic resonance imaging (MRI) done within 1 month before transplant. Of these, 14 explanted livers underwent WLHE retrospectively and prospectively by remnant whole explanted liver. This study was approved by the local Institutional Review Board, and written informed consent was obtained from all patients.

Patient characteristics. All 14 patients had liver cirrhosis classified as B or C

stage by Child-Pugh classification. The etiology in these cases was hepatitis C virus (HCV) infection in 8 patients and hepatitis B virus (HBV) in 6 patients. There were 6 females and 8 males, with a median age of 57 years (range, 48–61 years). The median values of alpha-fetoprotein (AFP) and protein-induced vitamin K antagonists II (PIVKAII)) were 30.25 ng/ml (range, 0.8-806.1) and 23 μg/ml (range, 6-247). The clinical characteristics of the 14 patients are summarized in Table 1.

Liver transplantation and preoperative therapy for HCC. In all 14 patients, LDLT had been performed using a right lobe graft in 11 patients and a left lobe graft in 3 patients. The median follow-up period was 30.1 months (range, 0.53-48.5 months). In 11 patients (78.5%), pretreatment for HCC was performed prior to liver transplantation, which consisted of chemolipiodolization in 6 cases, radiofrequency ablation (RFA) or percutaneous ethanol injection therapy (PEIT) in 4 cases, and chemolipiodolization with PEIT in 1 case. Based on the imaging findings, all HCCs were considered to be within the M-C.

Whole liver histological examination (WLHE). After explantation, the cirrhotic livers were fixed in formalin for 48 hours. The livers were then sectioned at 5-7 mm intervals, and each section was carefully inspected and mapped. All

sections were embedded in paraffin, and all slides were made from the paraffin-embedded material and routinely stained with hematoxylin and eosin. The median total number of slides for each patient was 116.5 (range, 64-185 slides). All slides were examined by an experienced pathologist (co-author S.O.). The pathological diagnoses and analyses were made according to the fourth edition of *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer,* published by the Liver Cancer Study Group of Japan (LCSGJ).

Statistical analysis. A statistical comparison of categorical variables was performed using the Mann-Whitney U test and Chi-square (χ^2) test. Results were considered statistically significant when the p values were less than 0.05.

Results

At the time of LDLT, there was no evidence of extrahepatic cancer spread in any of the patients. Preoperative imaging findings showed 4 patients with solitary HCC, 5 patients with double HCCs, 1 patient with triple HCCs, and 4 patients with no viable HCCs. In 3 patients, viable HCCs

had completely disappeared by the time of the preoperative treatment. Eight patients had a local recurrence or another new lesion in the liver based on imaging before LDLT. All patients met the M-C with a solitary nodule <5 cm in size or 3 nodules <3 cm for multi-nodular HCC.

Detection of HCCs by WLHE. In 9 patients (64.3%), undetectable nodules were found after WLHE, and 4 patients (28.6%) had preoperatively detectable nodules but no new lesions (Fig. 1). In 9 cases, small HCCs that could not be detected by the current imaging modalities were identified only by pathological examination. One patient (7.1%) (case 12) had a decreased number of HCCs compared to the number determined from preoperative imaging. The distribution of preoperatively undetectable nodules, which was based on segmental anatomy of the liver, was as follows: 4 nodules in segment 2, 4 nodules in segment 3, 7 nodules in segment 4, 1 nodule in segment 5, 6 nodules in segment 6, 3 nodules in segment 7, and 9 nodules in segment 8 (Fig.1). After WLHE, 5 out of the 14 patients (35.7%) were beyond the M-C. When we compared the results for the number and largest size of HCCs between imaging and WLHE, the largest size of HCCs was not altered

by WLHE, but the number of HCCs was increased by WLHE (Fig. 2). This was because small HCCs that could not be preoperatively detected by imaging were found in the cirrhotic liver by whole liver investigation.

Characteristics of preoperatively detectable HCCs. A total of 49 nodules were found by WLHE (Table 2). Fifteen nodules were found through preoperative images taken after histological examination, but 2 nodules detected preoperatively were not found in the explanted liver after WLHE. The median diameter of the 15 preoperatively detectable HCCs was 18 mm (range, 10-50 mm). There were 7 well-differentiated HCCs (46.7%) and 8 moderately differentiated HCCs (53.3%). The preoperatively detectable nodules showed expansive growth (11/15; 73.3%) with fibrous capsules (60%) (Table 2). Only two nodules (13.3%) showed microscopic vascular invasion surrounding the main tumor in the detectable HCCs.

Characteristics of preoperatively undetectable HCCs. Thirty-four HCCs that were undetectable preoperatively were found by WLHE (Table 2). The median diameter of the nodules was 6 mm (range, 2-15 mm). The

undetectable nodules consisted of 25 well-differentiated HCCs (73.5%), 9 moderately differentiated HCCs (26.5%), and no poorly differentiated HCCs. The characteristic features of the preoperatively undetectable nodules included infiltrative growth (29/34; 85.3%), absence of fibrous capsules (32/34; 94.1%), and absence of microscopic vascular invasion (32/34; 94.1%).

Pathological comparison of preoperatively undetectable and detectable HCCs. By comparing the pathological features of undetectable and detectable HCC based on the preoperative imaging results, we examined the relationship between tumor size and each of tumor differentiation, tumor growth, and the presence of fibrous capsules (Fig. 3-5). No significant difference in differentiation or growth was found between the preoperatively detectable and undetectable tumor size. However, we found significant differences in the presence of fibrous capsules between the undetectable and detectable HCCs (Fig 4).

Recurrence of HCCs. In the follow-up period (median follow-up, 30.1 months), there was no recurrence in any patient after LDLT.

Discussion

In this study, we demonstrated a discrepancy in the number of HCCs determined from preoperative imaging studies and the number determined from histological measurements using WLHE. It is especially important to note that multicentric HCCs were diffusely present in the end-stage cirrhotic liver, which is clinically significant since local therapy for only those HCCs that are detectable might not be a practical curative procedure in such cirrhotic livers. In addition, this study demonstrated that even HCCs within the M-C, which are usually regarded as early HCCs, can coincide with much earlier HCCs in the severely cirrhotic liver. This finding has not been demonstrated in autopsied livers from patients who died of advanced HCC.

In the present study, the accuracy of preoperative diagnosis for HCC was 28.6%, which was rather low compared to other reports (9-16). However, other studies used histological examination only for those nodules that were suspicious based on imaging findings, and not for the whole liver. Moreover, MDCT tends to overdiagnose HCC, with a false-positive diagnosis rate as high as 8-11.8% (11, 12). In addition, in a previous study MDCT could

detect only 61% of lesions smaller than 2 cm and 93.6% of lesions 2 cm or larger (12). Since our study demonstrated that the median diameter of preoperatively undetectable nodules was 6 mm (range, 2-15 mm), the overlooked lesions in previous studies were definitely below the detectable range of CT.

Of the 14 patients preoperatively within the M-C, undetectable HCCs were found in 9 patients (64.3%), with the result that 5 patients (35.7%) exceeded the M-C after WLHE, mainly due to the increased number of HCCs. The diameters of the largest HCCs determined from WLHE were identical to preoperative measurements. Therefore, current imaging modalities, such as MDCT, are good at evaluating the size of viable large HCCs, but not for determining the number of small HCCs. The reason the small HCCs were not detected by the current imaging modalities might be that they were characterized by an absence of fibrous capsules and an unclear border between the cancerous lesions and normal liver tissue. Another possible reason for the failure of the imaging methods to detect small HCC might be that the blood supply to the early HCC came more from the portal vein than the hepatic artery as in the case of advanced HCC. The

distribution of undetectable HCCs did not show any special features; i.e., undetectable HCCs could be found in any segment in the cirrhotic liver, implying that MDCT did not have blind areas.

In this study, there was no recurrence of HCCs after LT during the median follow-up period of 30.1 months, although 2 patients showed vascular invasion in the explanted livers by microscopic examination. Thus, HCCs in cases of advanced cirrhotic liver could be a multicentric phenomenon, and undetectable HCCs might not affect the survival rate after LT. Therefore, in the era of MDCT, the M-C would seem to be too strict a set of criteria for determining whether or not LT is indicated (17-20). In other words, if we follow the M-C as an indication of LT for HCC, the recurrence rate of HCC should be very much minimal.

In conclusion, HCCs in the severely cirrhotic liver might be characterized by multicentric occurrence. In this study, there were no recurrent HCCs after LDLT. Thus, preoperatively undetectable HCCs might not be associated with recurrence of HCC after LDLT. However, a high rate of recurrence can be expected when local therapy is performed for HCC, even with early-stage HCC, as determined by the M-C.

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Table 1. Clinical characteristics of 14 patients with cirrhosis and hepatocellular carcinoma

Age (year) (median, range)	57 (48-61)
Sex (M/F)	8/6
Cause of cirrhosis	
HBV	6
HCV	8
Child-Pugh classification	
B	5
C	9
Alpha-fetoprotein (<10/10-100/>100 ng/ml)	3/7/3
PIVKAII (<40/40> μg/ml)	8/6
Pretransplantation treatment	
Chemolipiodolization	6
PEIT or RFA	4
PEIT + Chemolipiodolization	1
none	3

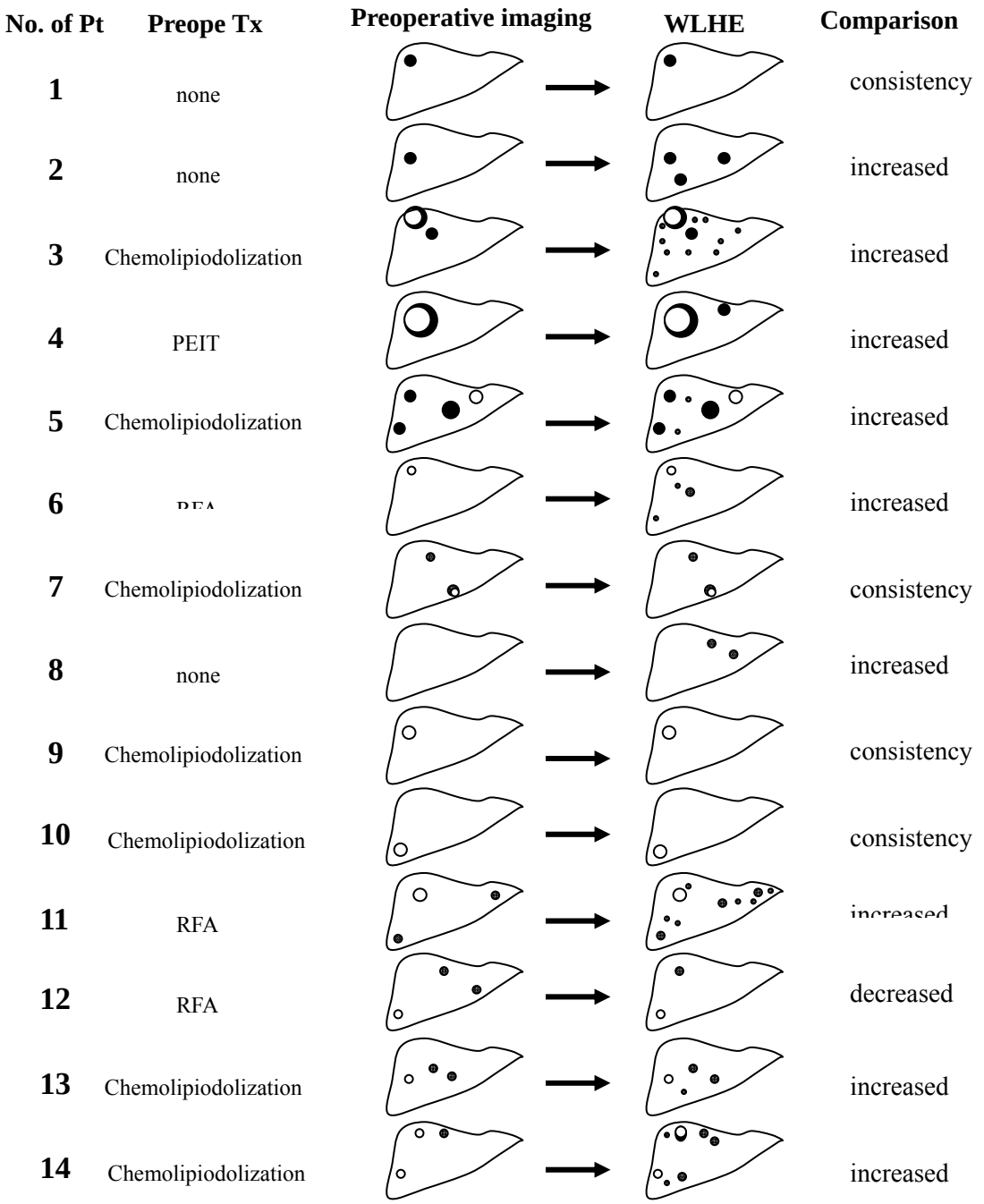
PIVKAII: protein-induced vitamin K antagonists II.
PEIT: percutaneous ethanol injection therapy; RFA: radiofrequency ablation.

Table 2. The characteristics of HCC on preoperative imaging study and postoperative histological study.

	HCC on imaging (n=15)	HCC only on WLHE (n=34)	
Diameter (median, range)(mm)	18 (10-50)	6 (2-15)	p=0.0000006
Differentiation			
Well	7 (46.7%)	25 (73.5%)	N.S
Moderate	8 (53.3%)	9 (26.5%)	N.S
Poorly	0	0	
Fibrous capsule	9 (60.0%)	2 (5.9%)	p=0.000028
Growth			
Expansive growth	11 (73.3%)	5 (14.7%)	N.S
Infiltrative growth	4 (26.7%)	29 (85.3%)	N.S
Microvascular invasion	2 (13.3%)	2 (5.9%)	N.S

N.S: not significant

Fig. 1. Detection of hepatocellular carcinoma using preoperative imaging and postoperative whole liver



● viable HCC: hepatocellular carcinoma,

○ Preoperative treatment with no viable HCC; PEIT: percutaneous ethanol injection therapy, RFA: radiofrequency ablation.

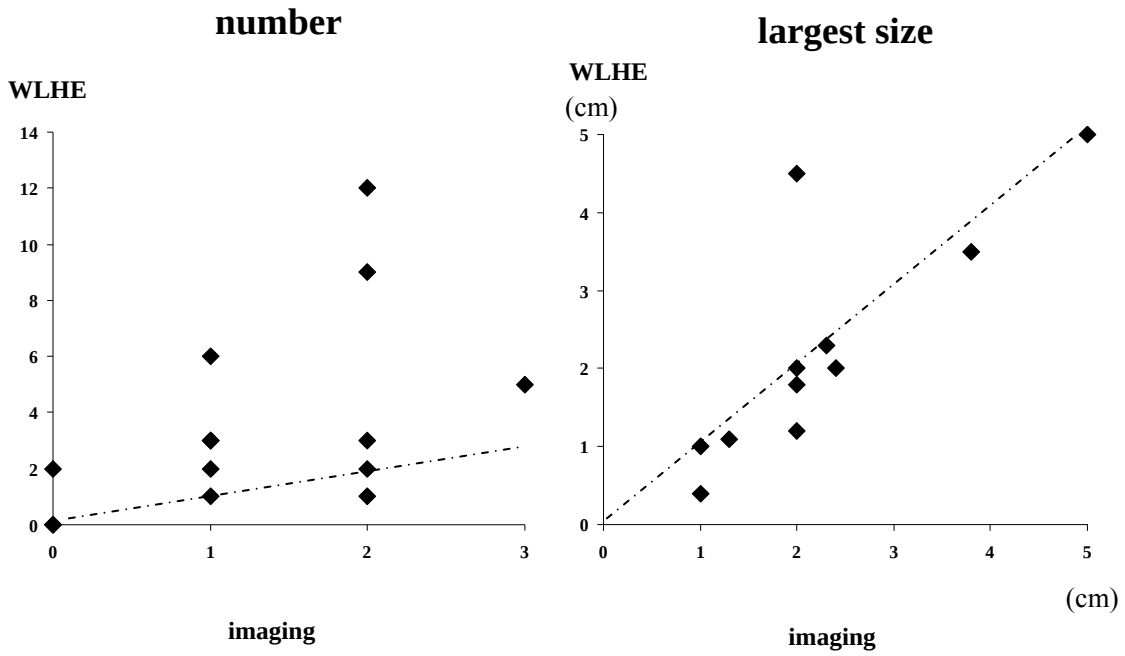


Fig. 2. The relationship of in size and in number between imaging and WLHE

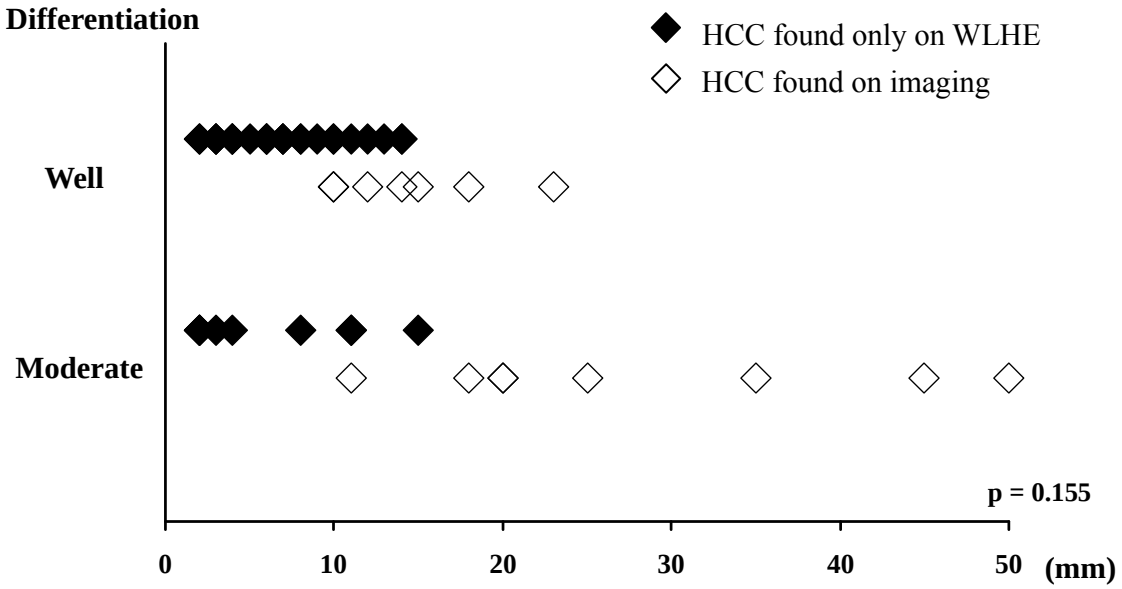


Fig. 3. The relationship between differentiation and size

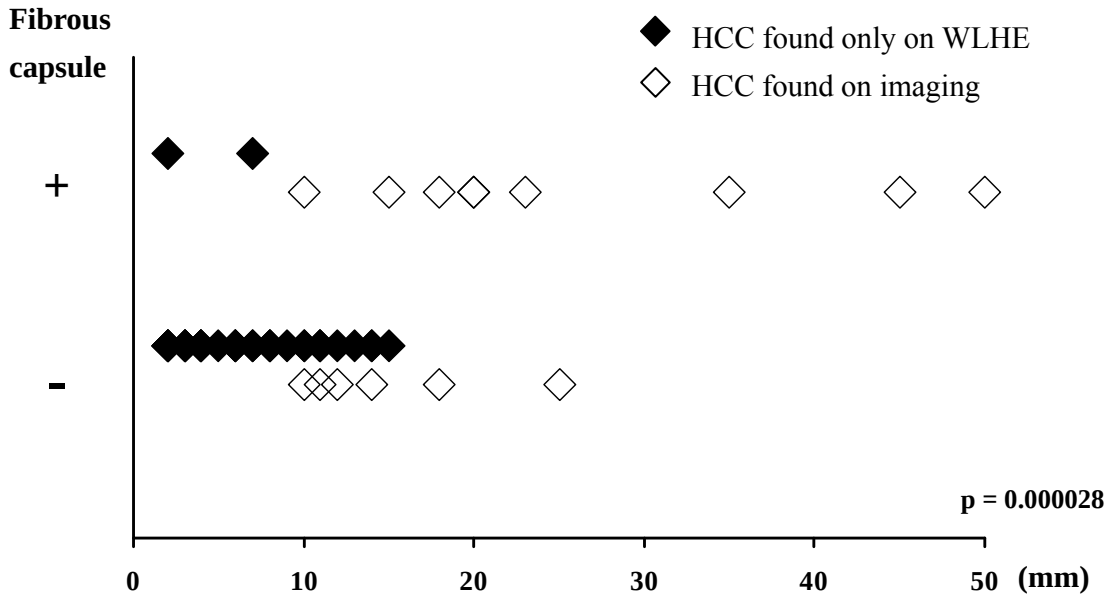


Fig. 4. The relationship between fibrous capsule and size

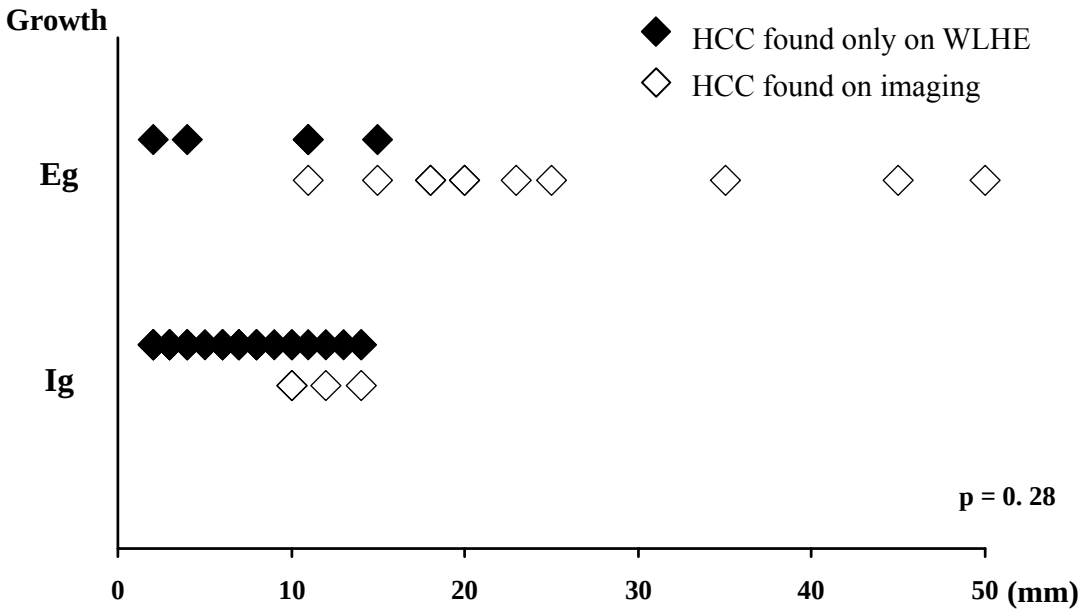


Fig. 5. The relationship between growth and size

Eg: Expansive growth; Ig: Infiltrative growth

Figure legends

Fig. 1. Detection of hepatocellular carcinoma using preoperative imaging and postoperative whole liver histological examination

Fig 2. The relationship of in size and in number between imaging and WLHE

Fig. 3. The relationship between differentiation and size

Fig 4. The relationship between fibrous capsule and size

Fig 5. The relationship between growth and size

Eg: Expansive growth; Ig: Infiltrative growth