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INTRAOPERATIVE ULTRASOUND FOR PREDICTION OF HEPATOCELLULAR CARCINOMA BIOLOGICAL BEHAVIOR: PROSPECTIVE COMPARISON WITH PATHOLOGY

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LIST OF ABBREVIATIONS:

HR: hepatic resection

HCC: hepatocellular carcinoma

MI: microinvasion

MI-HCC: microinvasive hepatocellular carcinoma

MRI: magnetic resonance imaging

IOUS: intraoperative ultrasound

BCLC: Barcelona Clinic Liver Cancer

EASL: European Association for the Study of the Liver

MELD: Model for end-stage liver disease

AFP: alpha-fetoprotein

ROC: Receiver operating characteristics

AUC: Corresponding areas under the ROC curve

CI: confidence intervals

PPV: positive predictive value

NPV: negative predictive value

PLR: positive likelihood ratios

IR: interquartile range

HCV: Hepatitis C virus

HBV: Hepatitis B virus

AST: Aspartate aminotransferase

ALT: alanine aminotransferase

CHE: cholinesterase

ALP: alkaline phosphatase

Diam: diameter

LPS: laparoscopic

OR: odds ratio

RFA: radiofrequency ablation

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ABSTRACT

Background and aims: Preoperative prediction of both microinvasive hepatocellular carcinoma and histological grade of hepatocellular carcinoma is pivotal to treatment planning and prognostication. Aim of the study was to evaluate whether some intraoperative ultrasound features correlate with both the presence of same histological patterns and differentiation grade of hepatocellular carcinoma on the histologic features of the primary resected tumor.

Methods: All patients with single, small hepatocellular carcinoma that underwent hepatic resection were included in this prospective double-blind study: the intraoperative ultrasound patterns of nodule were registered and compared with similar histological features.

Results: 179 patients were enclosed in this study: 97 patients (54%) (34% in HCC ≤2 cm) had a microinvasive hepatocellular carcinoma at ultrasound examination, while 82 cases (46%) (41% in HCC ≤2 cm) at histological evaluation. Statistical analysis showed that diameters ≤2 cm, presence of satellites and microinvasive hepatocellular carcinoma at ultrasound examination were the variables with the strongest association with the histologic findings. In the multivariate analysis, the vascular micro-infiltration and infiltrative hepatocellular carcinoma aspect were independent predictors for grading.

Conclusions: In patients with liver cirrhosis and hepatocellular carcinoma, the prevalence of microinvasive hepatocellular carcinoma is high, even in cases of HCC ≤2 cm. Intraoperative ultrasound findings strongly correlated with histopathologic criteria in detecting microinvasive patterns and are useful to predict neoplastic differentiation. The knowledge of these features prior to treatment are highly desired

(this can be obtained by an intraoperative ultrasound examination), as they could help in providing optimal management of patients with hepatocellular carcinoma.

KEYWORDS: liver cirrhosis, hepatic resection, liver transplantation, radiofrequency ablation, vascular microinfiltration, hepatocellular carcinoma

Key points box:

- Disease recurrence after hepatic resection for hepatocellular carcinoma might be predicted by pathological findings such as differentiation degree, presence of satellites and the existence of vascular microinfiltration.
- However, grade of hepatocellular carcinoma and aggressive patterns can be detect on surgical specimen.
- This study shows that intraoperative ultrasound examination can intraoperatively identify ultrasound features of aggressive tumors
- These patterns could influence clinical practice modifying the type of hepatic resection or the choice of ablation technology. Furthermore, they could be used to identify patients at high-risk for recurrence, and may improve the selection of patients undergoing transplantation

INTRODUCTION

Disease recurrence after hepatic resection (HR) for hepatocellular carcinoma (HCC) might be predicted by pathological findings such as differentiation degree, presence of satellites and the existence of vascular microinfiltration. These features could allow patients to be shortlisted for liver transplantation without proven malignant disease (1-3). The presence of microinvasion (MI) defined as portal venous, hepatic vein, bile duct infiltration and/or intrahepatic metastasis (satellite nodules) is the best indicator of poor prognosis after HR and transplantation; in fact, in this study of Yamashita et al (4), the recurrence-free survival of the MI-positive group after HR was significantly worse than that of the MI-negative group. Furthermore, the histological grade of HCC is a significant prognostic factor after surgery and high-grade HCC was found to be an independent predictor of vascular micro-infiltration (5-9).

Therefore, preoperative prediction of both microinvasive hepatocellular carcinoma (MI-HCC) and histological grade of HCC is pivotal for treatment planning and prognostication. In this high-risk group of patients, prevention and treatment of recurrence could improve the data of survival (10-11). However, histological grade of HCC and MI patterns are difficult to detect preoperatively with the current advanced imaging modality. Recent studies have shown that positron emission tomography (12), diffusion-weighted magnetic resonance imaging (MRI) (13) and contrast enhanced ultrasound (14) can be used to predict the histological grade and/or the presence of MI. Furthermore, one study (15) showed that the use of noninvasive radiologic criteria can predict biology of HCC accurately and correlated radiologic patterns directly to outcome. Our recent study (15) suggested that HCC recurrence is associated with HCC nodules presenting more aggressive biologic behavior and

these patterns could be identified by the intraoperative ultrasound (IOUS) evaluation: IOUS remains as the best real-time imaging method for evaluation of HCC nodules during laparoscopic and open liver surgery

In this prospective, double-blind study, we prospectively classified, for the first time to our knowledge, pattern combinations for each primary HCC nodule obtained by IOUS during HR. Then, we investigated whether these IOUS patterns correlate with both the presence of same histological patterns and differentiation grade of HCC on the histologic features of the primary resected tumor.

PATIENTS AND METODHS

Patients' characteristics

All patients had been assessed for disease staging with a pre-established protocol until 2000 (16); Barcelona Clinic Liver Cancer (BCLC) staging was retrospectively assessed in all patients enrolled before its accessibility (17).

A multidisciplinary team, which includes surgeons, radiologists, and hepatologists determined patient's eligibility for an invasive treatment. Criteria for staging and treatment evolved over-time. The diagnosis and staging of HCC were based on the appropriate imaging studies including triple-phase computed tomography (CT) and/or MRI according to the Barcelona-2000 European Association for the Study of the Liver (EASL) Conference, and histological assessment as required (18). Eligibility for liver transplantation (according to age, etiology, Child-Pugh and MELD score (Model for end-stage liver disease)) or HR was evaluated. Until 2012 (when the modified BCLC therapeutic algorithm was published (19)), HR was proposed

according to BCLC and AASLD guidelines: patients who had a single lesion can be offered surgical resection if they had cirrhosis with preserved liver function. Portal hypertension was not considered a contraindication in any case (20). Differing from the BCLC treatment protocol (19), in the time interval of this study we did not consider nodule size and number as absolute exclusion criteria from surgical treatment.

In this series, the residual liver function was evaluated using the Child-Pugh classification (21) and MELD (model for end-stage liver disease) score (22). Upon referral, laboratory tests including complete blood cell count, coagulation profile, liver functions, plasma levels of alpha-fetoprotein (AFP), and a chest x-ray were performed.

This study was approved by Institutional Review Board at Milan University. The patient records were anonymized and de-identified prior to analysis, and informed consent to storage the clinical data of patients with HCC was obtained from each participants.

IOUS evaluation

All patients underwent IOUS examination (Aloka Alfa 10; Aloka Co, Tokyo) during the operation. For laparotomic IOUS, T-shaped linear-type US probe was used at frequencies of both 5 MHz and 7.5 MHz, while for laparoscopic IOUS we used a laparoscopic ultrasound probe with a flexible tip, 10 mm in diameter and 50 cm in length and a 5 - 7.5 MHz linear-array transducer side-mounted near the tip of the shaft. IOUS image evaluation was performed by two blinded surgeons with more

than 10 years of experience using IOUS (R.S., M.B.), both of whom reviewed the sonographic images recorded on videotape and magneto-optical disks. In cases of discrepancies, the reviewers assessed the saved images together and reevaluated their findings to reach an agreement. They were unaware of the findings from the pathologic data during imaging evaluation. From the baseline IOUS features of the primary HCC nodule (15, 23), the surgeon was requested to classify the IOUS patterns according: diameter (registering also cut-off $\leq$20 mm), echogenicity (hyperechoic or hypoechoic), echotexture (homogeneous or inhomogeneous), nodular aspect (well-defined nodule versus infiltrative aspect) presence of pseudocapsule (hypoechoic or echogenic rim), capsular interruption (interruption of pseudocapsule + infiltrative aspect), mosaic architecture, nodule-in-nodule appearance, presence of satellites (identification of small nodules within 2cm away from HCC lesion) (Fig. 1A) and vascular/biliary micro-infiltration (either presence of vascular/biliary encasement or strict contact between vascular/biliary wall and nodule margins) (Fig 1C-1E). Number of HCC nodules was also considered: in this case, IOUS pattern has been determined for the largest nodule. According the histological definition proposed by Yamashita (4), MI-HCC was defined as a tumor with vascular/biliary infiltration and/or the presence of satellites.

Histopathologic evaluation

The pathology reports collected prospectively after surgery were used as a reference of standard for this study. All lesions were confirmed as HCC by surgical excision. Pathologists with hepatobiliary expertise who were blinded to the IOUS findings reviewed all histopathologic specimens. Tumor grade was scored using the modified

nuclear grading scheme outlined by Edmondson and Steiner (24): specifically, modified Edmondson–Steiner grades 1 and 2 were defined as well-differentiated, and grades 3 and 4 as moderately- and poorly-differentiated. In all cases, grade was defined by the poorest degree of differentiation identified within the tumor upon pathologic analysis of the entire specimen.

For the comparison with the IOUS patterns already described, the pathologist was requested to classify the specimen according: diameter (registering also cut-off ≤20 mm), presence of pseudocapsule versus infiltrative growth, capsular invasion (interruption of pseudocapsule and/or infiltrative aspect), nodule-in-nodule appearance, and vascular micro-infiltration (defined as presence of tumor emboli in a portal radicle vein, large capsular vessels or in a vascular space (25)). As described by Yamashita (4), MI-HCC was defined as vascular/biliary micro-infiltration (the presence of tumor cells within either a vascular space or a biliary duct that was visible only on microscopy) (Fig. 1D–1F) and/or the presence of satellites (Fig. 1B). Presence of cirrhosis was also confirmed on the surgical specimen.

Statistical analysis

The primary objective was to evaluate the ability of IOUS to depict the same pathological characteristics of HCC. The secondary objective was to determine whether IOUS patterns could be used to differentiate histopathologic grade of HCC. Data following a normal distribution were expressed as mean ± standard deviation. If data were non-parametric, median and interquartile range values were reported. Comparison of continuous variables between and within groups was performed by the Mann-Whitney U test and the Wilcoxon matched pairs test. Categorical variables

were analyzed by the Pearson's chi-square test. Independent risk factors were identified by logistic regression. Factors significant at a $p<0.10$ in the univariate analyses were included in a multivariate analysis.

Receiver operating characteristics (ROC) analyses were further constructed to determine the potential diagnostic performance for detecting the presence of MI. Corresponding areas under the ROC curve (AUC) with 95% confidence intervals (95% CI) were calculated. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) and likelihood ratios (PLR) were calculated for IOUS and histological evaluation using cross tabulations. For PLR, values above 10 indicate high predictive power, whereas values between 5 and 10 indicate good power; values below 5 indicate low predictive power and values around 1 indicate that no useful information for ruling the diagnosis in or out was produced. Agreement between IOUS patterns and surgical pathology were also reported using Cramer's V method (the value of the level of association was interpreted as follows: 0.00, no relationship; 0.00–0.10, not generally useful; 0.10–0.20, weak; > 0.20–0.25, moderate; > 0.25–0.30, moderately strong; > 0.30–0.35, strong; > 0.35–0.40, very strong; > 0.40–0.45, worrisomely strong; > 0.45–9.99, redundant; and 1.00, perfect relationship).

Initial evaluation, IOUS and histological data were collected in a dedicated database (FileMaker Pro for Macintosh; FileMaker Inc., Santa Clara, CA, USA) and subsequently analyzed (Intercooled Stata 14.1 for Macintosh, Stata Corp., College Station, TX, USA).

RESULTS

We enrolled patients who underwent curative HR for HCC at our University Hospital between January 2005 and March 2016. From a total of 285 resected patients with HCC, 179 patients were enclosed in this study. Patients with HCC diameter higher than 5 cm (62 patients) and with evidence of vascular encasement at preoperative imaging (11 patients) were excluded. The remaining 33 patients did not have a complete IOUS or pathologic database schedule. The baseline characteristics of the enrolled patients are summarized in Table 1.

In all patients IOUS has been accomplished: in 61 patients a laparoscopic IOUS probe was used, while a T-shaped probe was used in 118 subjects. New unsuspected lesions have been found in 23 patients (13%): in 10 cases (43% of the patients with new nodules) they were contiguous to primary HCC (satellite nodules), while in 13 cases (57%) they were located in other segments. For patients with new nodules contiguous to primary lesion, a IOUS-guided HR including these satellites has been accomplished, while for the others, we performed in 1 case (nodule in a contiguous segment) an enlarged HR, in 1 other case a second HR (nodule in other segment), in 6 cases an ethanol injection (nodule with a diameter less than 1 cm) and in the remaining 5 patients a RFA.

The IOUS and histological data of the patients' cohort are summarized in Table 2. After IOUS examination, MI-HCC was defined by the presence of biliary/vascular micro-infiltration and satellites in 20 cases (21%), by the only presence of vascular micro-infiltration in 55 cases (56%) and by the only presence of satellites in 22 cases (23%). Twenty-six cases out of the 54 with vascular micro-infiltration had vascular encasement with micro-thrombosis, while the other 28 cases showed only a strict

contact between HCC nodule and vessel wall. After histopathologic examination, MI-HCC was defined by the presence of biliary/vascular micro-infiltration and satellites in 27 cases (33%), by the only presence of vascular micro-infiltration in 35 cases (43%) and by the only presence of satellites in 20 cases (24%). IOUS-guided HR has been performed in these cases in order to include in the surgical specimen all satellites and/or biliary/vascular microinfiltration: for these reasons, anatomical HR has been performed in 75% of patients with MI-HCC in comparison to 62% of patients without MI-HCC (p=0.059).

Pathological examination revealed that 86 patients (48%) had a well-differentiated HCC, while the remaining 93 patients (52%) had a moderately/poorly-differentiated tumor. R0 resection was confirmed in all but 3 patients (1.7%), with median margin width of 5 mm (IR 2–10 mm).

Sensitivity, specificity, and positive and negative predictive values of IOUS are listed in Table 3: results of statistical analysis showed that diameters, presence of satellites and MI-HCC at IOUS examination were the variables with the strongest association with the histologic findings. Laparoscopic IOUS confirmed to have the same ability in comparison with open IOUS to identify the same variables with the best association with the histologic features (diameters, presence of satellites and MI-HCC) (Table 4). In the subgroup of patients with HCC diameter $\leq$2 cm, presence of satellites, vascular microinfiltration and MI-HCC were the variables with the strongest association with the histologic features (Table 5). Table 6 shows the IOUS image findings that had a correlation with histologic grade: the percentage of moderately/poorly differentiated HCC was significantly higher for infiltrative aspect of nodule, capsular interruption, vascular micro-infiltration and MI-HCC. In the multivariate analysis, only the vascular micro-infiltration (OR: 4.824; 95% CI: 1.919-

12.121; p=0.001) and infiltrative HCC aspect (OR: 2.313; 95% CI: 1.048-5.104; p=0.038) were independent predictors for HCC grading.

DISCUSSION

Several studies demonstrated that the presence of vascular micro-infiltration, satellites and/or high-grade HCC affects disease-free survival of patients with solitary HCC after curative HR (1, 4, 5, 7, 10, 11). Therefore, it is important to predict these features prior to treatment (in absence of a surgical specimen).

The use of preoperative tumor biopsy is controversial both for the risk of complications which is due to associated coagulopathy and the possibility of tumor spreading and for the inter-observer variability to define the histologic grade. In fact, few studies have evaluated, with conflicting results, the accuracy of percutaneous biopsy in comparison with the surgical specimen: HCC tumor differentiation is not homogeneous (8, 26, 27) and vascular micro-infiltration is difficult to detect at the pre-operative biopsy (7, 9).

Also due to these reasons, many factors and scoring systems evaluated by preoperative non-invasive procedures have been proposed to predict MI-HCC and/or HCC tumor differentiation (7, 28, 29). A study by Zhao et al. (30) demonstrated that serum AFP level >400 ng/L, serum GGT level >130 U/L, total tumor diameter >8 cm, and tumor number >3 were identified as independent predictors of vascular micro-infiltration, but this retrospective analysis needs a prospective validation. On the other hand, vascular micro-infiltration is impossible to rule out before surgery even with the more recent imaging procedures, such as ultrasonography, CT, and MRI,

because it is a microscopic lesion. Some Authors (31) identified “worrisome” imaging features, such as non-smooth tumor margins, peritumoral enhancement, TTPVI (“internal arteries” and “hypoattenuating halos”), and large tumor size, which were significant predictors of the presence of vascular micro-infiltration in HCC. However, the retrospective design of this study needs a prospective validation, while its single-center nature suggests substantial agreement among radiologists with a wide range of experience: in fact, other studies confirmed that there is no standard for MRI evaluation and the reproducibility of MRI features is still being investigated (32-34).

Other studies demonstrate that preoperative ^{18}F-FDG PET-TC is useful for predicting vascular micro-infiltration in small HCC (12). However, the low sensitivity of ^{18}F-FDG PET-CT for detection of primary HCC was a major concern in the prestudy planning. In particular, well-differentiated and early HCC may not be detected using FDG-PET (12, 35).

Yamashita et al (4) demonstrated that not only the presence of microinvasion such as portal venous, hepatic vein, bile duct infiltration, but also the presence of intrahepatic metastasis (satellites) is the best indicator of a poor prognosis after HR and transplantation. Therefore, MI-HCC may be considered an important entity (in line of principle superior to the only presence of vascular micro-infiltration) to determine the prognosis of patients submitted not only to HR or liver transplantation (4, 7, 36) but also to ablation therapies (37-39). Our study showed that IOUS definition of MI-HCC had a high agreement with the histological findings, such as the satellites detection and the correct measurement of HCC diameter (see table 3) and the type of IOUS examination (through either a laparoscopic or an open approach) did not influence these results (see table 4). This analysis is also valid in the subgroup of patients with HCC diameter $\leq$2 cm (see table 5). It is important to outline

that vascular microinfiltration showed weaker correlation with histological features: the main reason is intrinsic to IOUS definition of “vascular micro-infiltration” that is either presence of vascular encasement or strict contact between vascular wall and nodule margins. In this last case, it is possible that the contact with vascular wall does not represent a histologic encasement (Fig. 2). Furthermore, multivariate analysis showed that vascular micro-infiltration and infiltrative HCC aspect are related to tumor differentiation.

Therefore, IOUS examination during surgical procedures can identify more aggressive HCC nodule and this could influence clinical practice in different settings. For patients with HCC candidates to radiofrequency ablation (RFA), if preoperative clinical, biological and/or radiological features identified “worrisome” signs of aggressive behavior of HCC nodule, it could be preferable to propose a laparoscopic approach in order to accomplish IOUS evaluation. This examination is valid also for patients with HCC diameter $\leq$2 cm (4, 39).

During a laparoscopic thermo-ablation treatment, laparoscopic IOUS permits to identify MI-HCC tumors: in these cases, a microwave ablation instead of RFA could lead to a further clearance of possible residues of vascular micro-infiltration and satellite micronodules by ablating more viable tumor containing liver parenchyma and decrease probability of metastasis of the residual tumor cell by intrahepatic portal vein (40, 41).

During a HR, if a MI-HCC has been detected, it is possible to modify the surgical strategy and an anatomic HR or a partial HR with a wide tumor margin should be accomplished (36, 42). Anatomic resection can ideally eradicate MI-HCC confined to tumor-bearing portal tributaries (43).

Finally, during a surgical RFA of HCC, the IOUS evaluation of tumor patterns identified some HCC features, which could predict the HCC tumor differentiation: vascular micro-infiltration and infiltrative aspect at IOUS examination were well correlated with moderately/poorly differentiated HCC. This information could be used to identify patients at high-risk for HCC recurrence, and may improve the selection and survival of patients undergoing transplantation for HCC (3, 5, 6).

With recent improvements in technology, IOUS has now become an indispensable means of defining the extent of disease and resectability, and providing a guide to anatomic and nonanatomic HR and minimally invasive and ablative techniques. This study demonstrated that IOUS can also furnish important information about the prediction of HCC recurrences. However, the most important bias for the clinical application of these results is due to the fact that IOUS is an operator-dependent technique: with proper training, surgeons can accurately perform and interpret IOUS examinations and then use the results in the management of HCC patients.

In conclusion, HCC grading and vascular micro-infiltration is accepted worldwide as the two most important predictors of prognosis in patients with HCC both after HR and transplantation. The knowledge of these features prior to treatment are highly desired (and this can be obtained by the IOUS examination), as they could help in providing optimal management of HCC patients. In any case, these IOUS parameters could further increase prognostic precision in estimating the post-OLT risk of tumor recurrence, substantially improving the patient selection process. Further studies are necessary to evaluate if these IOUS features are directly predictive of intra-hepatic HCC recurrences.

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LEGEND OF FIGURES

Fig. 1: Intraoperative ultrasound images show HCC nodule with satellites (A), strict contact between vascular wall and nodule margin (C) and biliary microinfiltration (with dilated bile duct) (E). B: photograph of a resected specimen shows a nodular HCC with satellites, D: photomicrograph (H and E stain, x40) reveals vascular microinfiltration. F: photomicrograph (H and E stain, x40) shows HCC nodule with invasion of the bile duct and dilated bile duct

HCC: hepatocellular carcinoma; s: satellite; VMI: vascular microinfiltration; DBD: dilated bile duct

Fig. 2: intraoperative ultrasound imaging of strict contact between HCC nodule margins and vascular wall, which is considered as vascular microinfiltration (A); at histological examination, no vascular encasement is found (B and C) (photomicrograph, (H and E stain, x10 and x40)

HCC: hepatocellular carcinoma; VMI: vascular microinfiltration

	All patients (n=179)
Male sex	129 (72%)
Age (years) (median; IR)	69.6±8.6 (71; 65-75)
Cirrhosis etiology	
HCV	120 (67%)
HBV	31 (17%)
Other	28 (16%)
Child-Pugh Class A5	132 (74%)
Class A6	44 (24%)
Class B7	3 (2%)
MELD score (median; IR)	8.4±1.7 (8; 7-9)
BCLC [3]	
A1	100 (56%)
A2	32 (18%)
A3	14 (8%)

A4	33 (18%)
Esophageal varices	44 (25%)
Diabetes	58 (32%)
Previous HCC (already treated)	33 (18%)
HCC nodule: single /multiple	150 (84%)/ 29 (16%)
HCC lesion diameter (*mm*) (Median, IR)	27.5±10.4 (26; 20-34)
Platelet count (x100/mm3) (Median, IR)	132±56 (126; 89-169)
Total bilirubin (mg/dl) (Median, IR)	1.05±0.50 (1; 0.7-1.3)
Serum albumin (g/l) (Median, IR)	4.02±0.49 (4.06; 3.67-4.4)
Prothrombin time (INR) (Median, IR)	1.11±0.12 (1.09; 1.03-1.16)
AST (U/L) (Median, IR)	63+50 (47; 30-82)
ALT (U/L) (Median, IR)	66+54 (46; 30-84)
CHE (U/L) (Median, IR)	5976+2235 (5872; 4492-7228)
ALP (U/L) (Median; IR)	143+93 (110; 75-189)
Alpha-fetoprotein (ng/ml) (median, IR)	72+240 (7.5; 3.4–26.6)

Table 1. Baseline characteristics of the study population

IR: interquartile range; HCV: Hepatitis C virus; HBV: Hepatitis B virus; MELD: Model for end-stage liver disease; BCLC: Barcelona Clinic Liver Cancer; HCC: Hepatocellular carcinoma; AST: Aspartate aminotransferase; ALT: alanine aminotransferase; CHE: cholinesterase; ALP: alkaline phosphatase

Variables	IOUS	Histopathology
HCC diameter (mm) (median, IR)	26.7 ± 10.6 (24; 19-34)	26.2 ± 11.5 (25; 17-33)
Very Early (<2cm)	68 (38%)	70 (39%)
Pseudocapsule	130 (73%)	109 (61%)
Capsular interruption	120 (67%)	148 (83%)
Infiltrative growth	49 (27%)	76 (42%)
Nodule-in-nodule appearance	48 (27%)	10 (6%)
Satellites	42 (23%)	47 (26%)
Vascular micro-infiltration	75 (42%)	62 (35%)
MI-HCC	97 (54%)	82 (46%)
MI-HCC (HCC nodule <2cm)	23 (34%)	29 (41%)

Table 2: IOUS and histopathologic patterns

IOUS: intraoperative ultrasound, HCC: hepatocellular carcinoma, MI-HCC: microinvasive hepatocellular carcinoma

IOUS features	Sensitivity	Specificity	PPV	NPV	PLR	CRAMER V	Diagnostic OR (IC 95%)	AUCs (IC 95%)
Diam <20mm	91	83	89	85	5.3	0.7408	47.8 (17.9 – 132.3)	0.87 (0.82-0.92)
Pseudocapsule	40	81	57	81	2.1	0.2269	2.8 (1.3 – 5.8)	0.60 (0.53-0.67)
Capsular interruption	72	55	88	29	1.6	0.2130	3.1 (1.3 – 7.3)	0.63 (0.54-0.73)
Infiltrative growth	43	85	67	67	2.8	0.3091	4.2 (1.9 - 8.9)	0.63 (0.57-0.71)
Nodule-in-nodule appearance	80	76	17	98	3.4	0.2920	12.9 (2.4 – 92)	0.78 (0.65-0.92)
Satellites	72	94	81	91	11.9	0.6882	40.5 (14.2 – 120.5)	0.86 (0.79-0.92)
Vascular micro-infiltration	76	76	63	86	3.2	0.5003	9.9 (4.6 – 21.9)	0.74 (0.68-0.80)
MI-HCC	93	78	78	93	4.3	0.7424	45.8 (16.3-136.4)	0.85 (0.80-0.90)

Table 3: Intraoperative ultrasound image findings in relation to pathologic features of hepatocellular carcinoma

Diam: diameter; Mi-HCC microinvasive hepatocellular carcinoma; PPV: positive predictive value; NPV negative predictive value; PLR: positive likelihood ratio; OR: odds ratio; IC: interval of confidence; AUCs: area under curves

IOUS features	PLR		CRAMER V		Diagnostic OR (IC 95%)		AUCs (IC 95%)	
	LPS IOUS	Open IOUS	LPS IOUS	Open IOUS	LPS IOUS	Open IOUS	LPS IOUS	Open IOUS
Diam <20mm	22.2	3.7	0.7822	0.7221	121 (12-2924)	51 (14-216)	0.89 (0.82-0.97)	0.84 (0.77-0.92)
Pseudocapsule	2.9	1.7	0.3379	0.1656	4.6 (1.2-17.9)	2.1 (0.9-5.3)	0.65 (0.54-0.77)	0.58 (0.49-0-66)
Capsular interruption	1.2	1.9	0.0846	0.2753	1.6 (0.3-7.8)	4.2 (1.4-12.7)	0.55 (0.38-0-72)	0.67 (0.55-0.78)
Infiltrative growth	2.4	3.1	0.2696	0.3289	3.4 (0.9-12.7)	4.7 (1.8-12.4)	0.62 (0.51-0.74)	0.65 (0.56-0.73)
Nodule-in-nodule appearance	3.2	3.4	0.2364	0.3155	7.7 (0.5-235)	17.8 (1.9-410)	0.73 (0.4-1)	0.80 (0.66-0-95)
Satellites	4.9	5.4	0.4608	0.5543	12.9 (2.1-88.1)	15 (5.1-45.6)	0.77 (0.59-0.94)	0.78 (0.69-0.87)
Vascular micro-infiltration	4.6	2.2	0.4696	0.3769	10 (2.2-49)	5.2 (2.1-12.9)	0.73 (0.60-0-87)	0.69 (0.61-0.78)
MI-HCC	9.7	3.5	0.8257	0.6878	175 (16-4688)	40 (11-157)	0.93 (0.86-099)	0.84 (0.77-0.90)

Table 4: Intraoperative ultrasound image findings in relation to pathologic features of hepatocellular carcinoma for patients submitted to laparoscopic IOUS or open IOUS

Diam: diameter; Mi-HCC microinvasive hepatocellular carcinoma; LPS: laparoscopic; IOUS: intraoperative ultrasound; PLR: positive likelihood ratio; OR: odds ratio; IC: interval of confidence; AUCs: area under curves

IOUS features	Sensitivity	Specificity	PPV	NPV	PLR	CRAMER V	Diagnostic OR (IC 95%)	AUCs (IC 95%)
Pseudocapsule	39	78	67	53	1.8	0.1838	2.3 (0.7 – 7.7)	0.60 (0.47-0.75)
Capsular interruption	61	45	85	19	1.3	0.0516	1.3 (0.3 – 5.7)	0.52 (0.43-0.61)
Infiltrative growth	42	78	62	62	1.9	0.2190	2.7 (0.9 - 8.7)	0.62 (0.49-0.75)
Nodule-in-nodule appearance	33	83	8	96	1.9	0.0884	2.5 (0.1 – 40)	0.52 (0.44-0.61)
Satellites	71	98	92	91	36	0.7557	120 (11.4 – 3072.6)	0.92 (0.81-1)
Vascular micro-infiltration	57	93	67	89	7.7	0.5275	16.7 (3.2 – 97.5)	0.78 (0.63-0.92)
MI-HCC	87	95	91	93	19.2	0.8379	147 (18.6-1711.2)	0.92 (0.85-0.99)

Table 5: Intraoperative ultrasound image findings in relation to pathologic features of hepatocellular carcinoma $\leq$ 2 cm

MI-HCC microinvasive hepatocellular carcinoma; PPV: positive predictive value; NPV negative predictive value; PLR: positive likelihood ratio; OR: odds ratio; IC: interval of confidence; AUCs: area under curves

IOUS features	Well-differentiated	Moderately/poorly differentiated	p
Infiltrative aspect	14 (16%)	35 (38%)	0.001
Capsular interruption	51 (59%)	69 (74%)	0.034
Nodule-in-nodule appearance	20 (23%)	28 (30%)	NS
Satellites	22 (26%)	20 (22%)	NS
Vascular micro-infiltration	23 (27%)	52 (56%)	0.0001
MI-HCC	38 (44%)	59 (63%)	0.010

Table 6: Intraoperative ultrasound image findings in relation to pathologic grading of hepatocellular carcinoma

IOUS: intraoperative ultrasound; MI-HCC: microinvasive hepatocellular carcinoma