Contrast-enhanced ultrasound of intrahepatic cholangiocarcinoma: correlation with pathological examination

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Objective: To investigate the correlation between enhancement patterns of intrahepatic cholangiocarcinoma (ICC) on contrast-enhanced ultrasound (CEUS) and pathological findings.

Methods: The CEUS enhancement patterns of 40 pathologically proven ICC lesions were retrospectively analysed. Pathologically, the degree of tumour cell and fibrosis distribution in the lesion was semi-quantitatively evaluated.

Results: 4 enhancement patterns were observed in the arterial phase for 32 mass-forming ICCs: peripheral rim-like hyperenhancement ($n=19$); heterogeneous hyperenhancement ($n=6$); homogeneous hyperenhancement ($n=3$); and heterogeneous hypo-enhancement ($n=4$). Among the four enhancement patterns, the differences in tumour cell distribution were statistically significant ($p<0.05$). The hyperenhancing area on CEUS corresponded to more tumour cells for mass-forming ICCs. Heterogeneous hyperenhancement ($n=2$) and heterogeneous hypo-enhancement ($n=2$) were observed in the arterial phase for four periductal infiltrating ICCs. In this subtype, fibrosis was more commonly found in the lesions. Heterogeneous hyperenhancement ($n=1$) and homogeneous hyperenhancement ($n=3$) were observed in the arterial phase for four intraductal growing ICCs. This subtype tended to have abundant tumour cells.

Conclusion: The CEUS findings of ICC relate to the degree of carcinoma cell proliferation at pathological examination. Hyperenhancing areas in the tumour always indicated increased density of cancer cells.

Received 18 June 2011
Revised 19 October 2011
Accepted 25 October 2011

DOI: 10.1259/bjr/21653786

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Radiology

Intrahepatic cholangiocarcinoma (ICC) originates in the small bile duct and is grouped according to the International Classification of Diseases code, with hepatocellular carcinoma (HCC) being the primary liver tumour. It is the second most common primary liver tumour and is highly malignant. Although ICC is a relatively rare tumour, interest in this disease is rising because incidence and mortality rates for ICC are increasing steadily worldwide [1–5].

ICC is notoriously difficult to diagnose and is usually fatal, owing to its late clinical presentation and the lack of effective non-surgical therapeutic modalities. It tends to present with non-specific symptoms such as malaise, weight loss and abdominal pain. Most patients have unresectable disease at presentation and die within 12 months from the effects of cancer cachexia and a subsequent rapid decline in performance status.

According to growth characteristics, ICC is subcategorised into mass-forming, periductal infiltrating or intraductal-growing types by the Liver Cancer Study Group of Japan [6]. These subtypes show different biological behaviours and have different clinical outcomes.

Mass-forming ICC spreads between hepatocyte plates and expands via the hepatic sinusoidal spaces. It often invades the adjacent peripheral branches of the portal vein. Periductal-infiltrating ICC tends to spread along the bile duct wall via the nerve and perineural tissue of Glisson's capsule towards the porta hepatis. Intraductal-growing ICCs are usually small or polypoid and do not invade deeply into the submucosal layer, often spreading superficially along the mucosa surface. Characterisation of the tumours in terms of their growth pattern is necessary for optimal treatment planning and prognosis assessing. The prognosis for mass-forming and periductal-infiltrating cholangiocarcinoma is generally unfavourable, but is much better for the intraductal-growing type after surgical resection, and long-term patient survival can be expected [7, 8].

Contrast-enhanced ultrasound (CEUS) has been increasingly applied in liver imaging. By administration of ultrasound contrast agents, CEUS can display dynamic blood flow perfusion and microcirculation of liver lesions [9], similar to CT and MRI. In previous studies, CEUS had a similar diagnostic accuracy for ICC to CT and was suggested as an alternative diagnostic option when CT examination was not available for patients with iodine allergy or impaired renal function [10]. It was confirmed that CT and/or MRI findings of ICC were correlated with pathological findings; that is, the

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hyperenhancing areas always indicated a large number of tumour cells and the areas of delayed enhancement corresponded to fibrotic stroma at pathological examination. In addition, different morphological subtypes tended to exhibit distinct enhancement characteristics on CT [7, 8, 11–13]. On CEUS, besides the specific feature of peripheral rim-like hyperenhancement, diverse imaging findings of ICC were reported [9, 10, 14–17]. These different CEUS appearances may reflect the differences in pathological subtypes or components of ICC. The aim of this study was to investigate the correlation between the enhancement pattern of ICC on CEUS and pathological findings. This information may be useful for diagnosis, treatment planning and prognostic evaluation of ICC.

Methods and materials

Patients

The study was approved by the ethics committee of the First Affiliated Hospital of Sun Yat-Sen University and written informed consent was obtained from each patient prior to CEUS.

From March 2004 to April 2007, 59 patients with ICC underwent CEUS examination in the hospital. The diagnoses of all patients were confirmed by pathological examination. The specimens were obtained by ultrasound-guided percutaneous biopsy in 9 patients and by surgery in 50. Among the 50 surgery cases, tumours in 3 cases that were located in the left lobe of the liver (mean size, 8.1 ± 3.4 cm; range, 4.6–11.4 cm, measured via ultrasound) were unresectable owing to abdominal wall ($n=1$) or lesser omentum ($n=2$) infiltration. Partial hepatectomy was performed in 47 cases. Among them, seven patients had multiple lesions (two lesions in three patients, three lesions in one and more than five lesions in three); these patients were excluded because, in this retrospective study, it was uncertain whether or not the lesion observed via CEUS was identical to the one observed via pathological examination. 40 patients (21 males and 19 females: age range, 28–76 years; mean age, 59 ± 12 years) who had solitary lesions were included in our study for retrospective analysis.

According to the cholangiocarcinoma classification proposed by the Liver Cancer Study Group of Japan [6], the lesions were classified into one of the three groups as either a mass-forming type ($n=32$), in which the tumour is an apparent nodule in the liver parenchyma and the borders between the cancerous and non-cancerous portions are distinct; a periductal-infiltrating type ($n=4$), in which the tumour extends mainly along the peribiliary duct area and is elongated, spiculated or branchlike; or an intraductal-growing type ($n=4$), in which the tumour develops into the bile duct lumen and forms an intraluminal occlusive lesion. These classifications were based on the gross morphological findings of the surgical specimens.

CEUS examination

CEUS was performed with an ACUSON Sequoia™ 512 (Siemens Medical Solutions, Mountain View, CA) in 32

patients, with a 4V1 vector transducer (frequency range, 1.0–4.0 MHz). A contrast-specific mode of contrast pulse sequencing (Siemens Medical Solutions), which allowed continuous real-time observation (mechanical index range, 0.15–0.21), was applied. Eight patients were scanned with an Aplio XV (Toshiba Medical Systems, Tokyo, Japan) with a 375BT convex transducer (frequency range, 1.9–6.0 MHz). The contrast imaging mode was Contrast Harmonic Imaging (Toshiba Medical Systems) and the mechanical index range was 0.05–0.08. The contrast agent was SonoVue® (BR1) (Bracco, Milan, Italy), which was a sulphur hexafluoride-filled microbubble.

Two experienced radiologists with more than 3 years' experience in liver CEUS performed the scanning. The examinations were performed by both radiologists separately prior to biopsy or surgical resection. Both radiologists had received training in advance to ensure that all the procedures were performed in the same fashion. Before CEUS was undertaken, the radiologists were told that the patients already had focal liver lesions detected by previous imaging studies but were blind to the results. Firstly, the whole liver was scanned with baseline ultrasound (BUS) and the target lesion was determined. Then the imaging mode was changed to CEUS with the transducer kept in a stable position. The mechanical index settings and initial gain were adjusted to suppress the echoes from the tissue and minimise microbubble loss in the anatomical region of interest. Subsequently, a volume of 2.4 ml of SonoVue was administered with a bolus injection (within 1–2 s) through the antecubital vein, followed by a flush of 5 ml of normal saline using a 20-gauge cannula. The whole CEUS process was observed continuously for more than 240 s and all cine clips were stored digitally on a hard disk and subsequently transferred to a personal computer for retrospective analysis. After 240 s, the contrast agents washed out progressively and CEUS was stopped when the contrast agents were nearly invisible on the screen.

Image analysis

Two experienced readers, who had more than 3 years' experience in liver CEUS, assessed the CEUS images subjectively in consensus. The two readers were independent of the two who performed the ultrasound examination. Information about other imaging results, clinical presentations, laboratory tests and histopathology of the patients was hidden to the readers. The CEUS process was classified as arterial (8–30 s after the contrast agent injection), portal (31–120 s) or late (121–240 s) phase according to the European Federation of Societies for Ultrasound in Medicine and Biology guidelines for CEUS [9]. The enhancement levels (hyper-, iso- or hypo-enhancement, in comparison with adjacent liver parenchyma) and their dynamic changes along with the three phases were recorded. The enhancement patterns in the arterial phase were subcategorised as follows: peripheral rim-like hyperenhancement—a continuous contrast hyperenhancing ring at the periphery of the lesion; heterogeneous hyperenhancement—enhancement with different levels, with the highest level being

hyperenhancement; homogeneous hyperenhancement—hyperenhancement with similar levels; and heterogeneous hypo-enhancement—enhancement with different levels, with the highest level being hypo-enhancement. The intratumoral blood vessels displayed during the arterial phase were also evaluated.

Histopathological examination

All 40 resected specimens were fixed in 10% formalin and >4 thin tissue sections were cut. For the mass-forming type, two separate regions were chosen to cut in the largest dimension of the specimens: the peripheral portion and the central portion, avoiding any large vessels and large areas of necrosis or bleeding. Subsequently, they were embedded in paraffin, cut into 4 µm thick slices and stained with haematoxylin and eosin. A pathologist with 6 years' experience in liver disease who was blinded to the CEUS and other imaging findings reviewed the histological specimens retrospectively. The stained sections were observed under a light microscope at ×200 magnification. The degree of tumour cell and fibrosis distribution in the lesion was semi-quantitatively evaluated with reference to previous reports [18, 19], using a scoring system based on a 3-point scale in 10 randomly chosen fields. This was defined as follows: 1, little (<10% of field); 2, moderate (10–50%); 3, abundant (>50%). The CEUS enhancement patterns and the densities of tumour cells and fibrosis in three types of ICC were compared.

Statistical analysis

The quantitative data were expressed as mean ± standard deviation. The comparison between the CEUS results and pathological appearance was tested using the χ^2 test. A *p*-value of <0.05 was considered statistically significant. The statistical analyses were performed using the SPSS® v. 13.0 software package (SPSS Inc., Chicago, IL).

Results

Ultrasound and contrast-enhanced ultrasound appearances

In total, 40 lesions were observed. The mean size was 6.7 ± 3.1 cm, with a range from 2.0 to 15.5 cm. The depth from the body surface to the bottom of the lesion ranged from 3.7 to 16.2 cm (mean, 8.2 ± 2.8 cm).

Mass-forming intrahepatic cholangiocarcinoma

There were 32 (80%) mass-forming ICCs (mean size, 7.1 ± 3.1 cm; range, 2.1–15.5 cm). On BUS, hyper, iso- and hypo-echogenicity were seen in 5 (15.6%), 5 (15.6%) and 22 (68.8%) of the 32 lesions, respectively. 10 (31.2%) lesions were regular and 22 (68.8%) were irregular. 24 (75.0%) lesions were well defined and 8 (25.0%) were ill-defined. 11 (34.4%) lesions showed peripheral bile duct dilation around the tumour and 7 (21.9%) showed stones in the masses.

After administration of contrast agent, 4 enhancement patterns were observed in the arterial phase as follows: peripheral irregular rim-like hyperenhancement in 19 (59.4%) lesions, heterogeneous hyperenhancement in 6 (18.8%), homogeneous hyperenhancement in 3 (9.4%) and heterogeneous hypo-enhancement in 4 (12.5%). 9 (28.1%) lesions displayed intralesional blood vessels in the arterial phase (Figures 1–4).

In the portal phase, 1 (3.1%) mass-forming ICC showed iso-enhancement; the other 31 (96.9%) ICCs showed hypo-enhancement. During the late phase, all 32 (100%) lesions faded out to show hypo-enhancement (Figures 1–4).

Periductal-infiltrating intrahepatic cholangiocarcinoma

Periductal-infiltrating ICCs were observed in 4 (10%) lesions (mean size, 6.0 ± 3.5 cm; range, 3.0–9.8 cm). On BUS, 1 (25.0%), 1 (25.0%) and 2 (50.0%) lesions showed hyper, iso- and hypo-echogenicity, respectively. All (100.0%) of these were irregular and ill-circumscribed. 2 (50.0%) showed vicinal bile duct dilation and 1 (25.0%) showed stones in the lesion.

On CEUS, heterogeneous hyperenhancement in 2 (50.0%) lesions and heterogeneous hypo-enhancement in 2 (50.0%) lesions were observed in the arterial phase. The 2 (50.0%) hyperenhancing lesions displayed intralesional blood vessels. All four lesions showed hypo-enhancement in both the portal and late phases.

Intraductal-growing intrahepatic cholangiocarcinoma

Intraductal-growing ICCs were observed in 4 (10%) lesions (mean size, 3.9 ± 1.3 cm; range, 2.0–4.8 cm). On BUS, all of the four intraductal-growing ICCs were hyperechoic. 3 (75.0%) lesions were irregular and 1 (25.0%) was regular. 3 (75.0%) lesions had well-circumscribed boundaries and 1 (25.0%) had an ill-circumscribed boundary. Local bile duct dilation was observed around all (100.0%) lesions and a stone was observed in 1 (25.0%) lesion.

On CEUS, heterogeneous hyperenhancement in 1 (25.0%) and homogeneous hyperenhancement in 3 (75.0%) lesions were observed in the arterial phase. No lesion showed intralesional blood vessels. All four lesions showed hypo-enhancement in both the portal and late phases.

Histopathological findings

Mass-forming ICCs were adenocarcinomas with variable differentiation: 6 (18.8%) of 32 lesions were well differentiated; 11 (34.4%) were moderately differentiated; and 15 (46.9%) were poorly differentiated. Glandular structures grew in cellular nests that were separated by variable fibrous stroma. Carcinoma cells were more actively proliferating in peripheral portions of the tumour, while the central portions were more sclerotic

Figure 1. Intrahepatic cholangiocarcinoma. (a) Baseline ultrasound shows an ill-defined iso-echoic mass (arrows) in the right lobe of the liver. (b) Contrast-enhanced ultrasound shows peripheral rim-like hyperenhancement (arrowheads) around the tumour (arrows) during the arterial phase, but hypo-enhancement in the centre portion. (c) In the portal phase, the whole lesion (arrows) shows hypo-enhancement. (d) The lesion (arrows) continues to show hypo-enhancement in the late phase. (e) Microscopically, pathological examination reveals abundant tumour cells in the peripheral portion of the tumour (haematoxylin-eosin stain, $\times 200$ magnification). (f) There are scarce tumour cells in the centre portion of the tumour and fibrosis is prominent (haematoxylin-eosin stain, $\times 200$ magnification).

and hypocellular. Central necrosis was present in 15 (46.9%) lesions.

Of the 4 periductal-infiltrating ICCs, 2 (50.0%) were well differentiated and the other 2 (50.0%) were moderately differentiated. Carcinoma cells infiltrated into the peribiliary glands, with considerable desmoplasia and thickening of the affected bile duct walls.

4 intraductal-growing ICCs were papillary adenocarcinomas histologically; 3 (75%) lesions were well differentiated and 1 (25.0%) was moderately differentiated. This type comprised innumerable frond-like infoldings of columnar epithelial cells. The tumours were confined within the mucosal layer of the bile ducts and did not invade deep into the submucosal layer.

Correlation of contrast-enhanced ultrasound enhancement patterns and pathological findings

The distribution of tumour cells and the scores in the peripheral and central portions for the mass-forming ICCs are presented in Tables 1 and 2. Among the four enhancement patterns, the differences in tumour cell

distribution were statistically significant ($p < 0.05$ at both the peripheral and the central portions); however, there were no significant differences in terms of fibrosis distribution ($p > 0.05$ at both the peripheral and the central portions). There was a trend that hyperenhancing areas of the lesion on CEUS corresponded to more tumour cells (Figures 1–4).

Of the periductal-infiltrating ICCs, one heterogeneously hyperenhanced lesion had a distribution of tumour cell score of 1; another had a score of 2. Both of these lesions had a distribution of fibrosis score of 3. The remaining two heterogeneously hypo-enhanced periductal-infiltrating ICCs were scored as 1 and 3, respectively, with regard to the distribution of tumour cells and fibrosis. In this subtype, fibrosis was more commonly found in the lesion.

The degree of tumour cell distribution in three homogeneously hyperenhanced intraductal-growing ICCs was scored as 3, and the degree of fibrosis distribution was scored as 1 in 1 (33.3%) and as 3 in 2 (66.7%) lesions. The remaining heterogeneously hyper-enhanced intraductal-growing ICC was scored as 2 with regard to the distribution of both tumour cells and

Figure 2. Intrahepatic cholangiocarcinoma. (a) Baseline ultrasound shows an ill-defined mass (arrows) with mixed echogenicity in the right lobe of the liver. (b) Contrast-enhanced ultrasound shows heterogeneous hyperenhancement (arrows) during the arterial phase. (c) In the portal phase, the lesion (arrows) becomes slightly hypo-enhancing. (d) The lesion (arrows) becomes hypo-enhancing in the late phase. (e, f) Microscopically, abundant tumour cells are present in both the peripheral portion (e) and the centre portion (f) (haematoxylin-eosin stain, $\times 200$ magnification).

fibrosis. In total, the lesions in this subtype tended to have abundant tumour cells.

Discussion

ICC arises from the bile ducts peripheral to the secondary bifurcation of the left or right hepatic duct. The role of imaging procedures in early detection and diagnosis is important. Before the introduction of CEUS, ultrasound was used only for screening in focal liver lesion detection; characterisation of the tumour usually required CT or MRI [7, 8].

An outstanding advantage of CT over BUS is that administration of the contrast medium enables CT to demonstrate the microcirculation perfusion of the tumour. For instance, the mass-forming type of ICC has a distinctive appearance on CT; that is, a thin, mild, incomplete rim-like contrast enhancement at the periphery and hypo-attenuating centres during both the arterial and the portal venous phases, but a mild or marked increase in tumour attenuation with progressive and centripetal filling of contrast medium in the delayed phase [7, 8, 12]. The involved bile duct walls in periductal-infiltrating tumours were diffusely thickened,

which showed hyperattenuation in the arterial phase, with higher attenuation during the portal and late phases. The bile ducts proximal to the tumour were dilated owing to the narrowed or obliterated duct lumens [7]. Intraductal tumours could appear as a hypo-attenuating mass in dilated bile ducts, with a very low enhancement relative to the liver.

In previous studies, four different enhancement patterns of ICC on CEUS were reported. These were peripheral irregular rim-like hyperenhancement, heterogeneous hyperenhancement, homogeneous hyperenhancement and heterogeneous hypo-enhancement [10, 14–17]. In the present study, the most common type of ICC was the mass-forming type (80%). The common appearance of mass-forming ICCs on BUS was a single hypo-echoic mass with irregular borders. On CEUS, 87.5% of them were hypervascular and 28.1% showed blood supply vessels within the tumour. The same four enhancement patterns were demonstrated in this type. Among them, peripheral irregular rim-like hyperenhancement was seen in 59.4% of lesions. This appearance corresponds well with the important pathological characteristic of ICC: marked fibrous stroma in the centre and abundant carcinoma cells at the edge. In the homogeneous or heterogeneous hyperenhancing ICCs, carcinoma cells

Figure 3. Intrahepatic cholangiocarcinoma. (a) Baseline ultrasound shows an iso-echoic mass (calipers) in the right lobe of the liver. (b) The lesion (arrows) shows homogeneous hyperenhancement during the arterial phase. (c) The lesion (arrows) becomes slightly hypo-enhancing during the portal phase. (d) The lesion (arrows) becomes hypo-enhancing during the late phase. (e, f) Microscopically, abundant tumour cells are present in both the peripheral portion (e) and the centre portion (f) (haematoxylin-eosin stain, $\times 200$ magnification).

were prominent in the centre as well as at the periphery. By contrast, in heterogeneous hypo-enhancing ICCs, only a small number of carcinoma cells were scattered at the edge and in the centre. It is well known that ultrasound contrast agents perform as blood pool tracers; accordingly, CEUS could provide information about the degree of lesion vascularity. Pathophysiologically, carcinoma cells would proliferate densely in the areas with abundant blood supply. Therefore, as was demonstrated in this study for mass-forming ICC, the hyperenhancing areas on CEUS correspond with abundant carcinoma cells, pathologically, and different distributions of carcinoma cells may display different CEUS enhancement patterns.

It was reported that the areas of delayed or prolonged enhancement in ICC at CT imaging, which corresponded to fibrotic stroma at histopathological examination [7, 8, 11, 12], could reflect the degree of fibrosis within the tumour. On the contrary, no lesion in this group showed delayed enhancement on CEUS, and there was no correlation between the CEUS enhancement patterns and the degree of fibrosis in ICC. The delayed enhancement characteristics of ICC seem to be due to the CT contrast material being rapidly cleared from the blood pool and its retention within the large interstitial space in the fibrous stroma of the tumour during

the late phase [11, 12]. However, ultrasound contrast agents have different pharmacokinetics. They are blood pool tracers and are confined to the intravascular space. Therefore, instead of implicating the degree of fibrosis of the tumour, CEUS demonstrates the real blood flow perfusion within the tumour.

Periductal-infiltrating ICC is pathologically identical to infiltrating hilar cholangiocarcinoma, but has a different location [7, 8]. It results in irregular narrowing of the involved bile duct and eventual obstruction. Four periductal-infiltrating ICCs in our study showed a scirrhous type of growth, with extensive stromal desmoplasia and sparse tumour cell infiltration. On BUS, instead of a well-defined mass, an irregular, confounding mixed-echoic area in the liver with ambiguous boundary may be found. On CEUS in our study, two lesions showed heterogeneous hypo-enhancement with no supplying vessels; the other two showed heterogeneous hyperenhancement and tumour vessels were visualised. The latter enhancement may have been associated with the fact that periductal-infiltrating tumours invaded the bile duct wall or hepatic parenchyma in close association with or involving surrounding periductal blood vessels, providing a relatively abundant vascular supply.

Figure 4. Intrahepatic cholangiocarcinoma. (a) Baseline ultrasound shows an ill-defined iso-echoic mass (arrows) in the left lobe of the liver. (b) The lesion (arrows) shows heterogeneous hypo-enhancement during the arterial phase. (c) The lesion (arrows) remains hypo-enhancing during the portal phase. (d) The lesion (arrows) continues to be hypo-enhancing during the late phase. (e, f) Microscopically, scarce tumour cells are present in both the peripheral portion (e) and the centre portion (f) (haematoxylin-eosin stain, $\times 200$ magnification).

Four intraductal-growing ICCs were found. The mass appeared specifically as a hyperechoic intraductal mass within focal dilated bile ducts, with an irregular and clear margin on grey-scale ultrasound. They presented as homogeneous or heterogeneous hyperenhancement in the arterial phase, and as hypo-enhancement in the portal and late phases. The corresponding pathological

findings were papillary adenocarcinoma, which is a low-grade malignancy that forms sessile or polypoid masses, consisting of innumerable papillary frond-like infoldings of proliferated columnar epithelial cells and fibrovascular cores. Owing to their growth pattern, the tumours spread superficially along the mucosa of the bile duct instead of invading adjacent tissues [7, 8].

Table 1. Correlation of Contrast-enhanced ultrasound enhancement pattern and pathological distribution of tumour cells in 32 mass-forming intrahepatic cholangiocarcinomas

Enhancement patterns	Distribution of tumour cells in the peripheral portion ^a			Distribution of tumour cells in the central portion ^a		
	Score 1	Score 2	Score 3	Score 1	Score 2	Score 3
Peripheral irregular rim-like hyperenhancement ($n=19$)	0 (0)	8 (42.1)	11 (57.9)	18 (94.7)	1 (5.3)	0 (0)
Heterogeneous hyperenhancement ($n=6$)	0 (0)	4 (66.7)	2 (33.3)	1 (16.7)	5 (83.3)	0 (0)
Homogeneous hyperenhancement ($n=3$)	0 (0)	1 (33.3)	2 (66.7)	1 (33.3)	0 (0)	2 (66.7)
Heterogeneous hypo-enhancement ($n=4$)	2 (50.0)	2 (50.0)	0 (0)	3 (75.0)	1 (25.0)	0 (0)
Total ($n=32$)	2 (6.3)	15 (46.9)	15 (46.9)	23 (71.9)	7 (21.9)	2 (6.3)
p -value	0.007 ^b			<0.001 ^b		

Data are expressed as number (percentage) unless otherwise indicated.

^aScore 1, little (<10% of field); score 2, moderate (10–50% of field); score 3, abundant (>50% of field).

^bIndicates statistically significant difference.

Table 2. Correlation of contrast-enhanced ultrasound enhancement pattern and pathological distribution of fibrosis in 32 mass-forming intrahepatic cholangiocarcinomas

Enhancement patterns	Distribution of fibrosis in the peripheral portion ^a			Distribution of fibrosis in the central portion ^a		
	Score 1	Score 2	Score 3	Score 1	Score 2	Score 3
Peripheral irregular rim-like hyperenhancement (n=19)	11 (57.9)	8 (42.1)	0 (0)	3 (15.8)	9 (47.4)	7 (36.8)
Heterogeneous hyperenhancement (n=6)	2 (33.3)	4 (66.7)	0 (0)	1 (16.7)	3 (50.0)	2 (33.3)
Homogeneous hyperenhancement (n=3)	2 (66.7)	1 (33.3)	0 (0)	1 (33.3)	2 (66.7)	0 (0)
Heterogeneous hypo-enhancement (n=4)	2 (50.0)	2 (50.0)	0 (0)	2 (50.0)	1 (25.0)	1 (25.0)
Total (n=32)	17 (53.1)	15 (46.9)	0 (0)	7 (21.9)	15 (46.9)	10 (31.2)
p-value	0.716			0.693		

Data are expressed as number (percentage) unless otherwise indicated.

^aScore 1, little (<10% of field); Score 2, moderate (10–50% of field); Score 3, abundant (>50% of field).

Peripheral irregular rim-like hyperenhancement and heterogeneous hypo-enhancement are specific enhancement patterns of the mass-forming type of ICC shown on CEUS. Some metastatic liver cancers may show rim-like hyperenhancement too, so exclusion of an extrahepatic primary site is the most important clue for discrimination. hypervascular ICCs should be distinguished from HCCs. Information such as the background of liver cirrhosis or higher serum α -fetoprotein values may be helpful [20]. On BUS, biliary sludge or muddy stones can produce intraluminal echoes that may be difficult to distinguish from intraductal growing ICCs. After injection of contrast agents, the presence of enhancement can eliminate the possibility of sludge or stones.

There were some limitations in this study. The case numbers of periductal-infiltrating ICCs and intraductal-growing ICCs were small, so it was difficult to reach a conclusion. Like other retrospective studies, there was limited control in the selection of the patient population. In addition, we did not investigate the relationship between CEUS enhancement appearances and microvessel density. Although there was no direct association between CEUS enhancement patterns and tumour cell distribution (because contrast agents were trapped only in the blood vessels and tumour cells were abundant in areas of rich vascularity), we showed that the CEUS findings correlated with tumour cell distribution. Another limitation was the use of two scanners that might have caused bias in image evaluation. However, both scanners worked under a low mechanical index; therefore, their influence was minimised. In addition, no objective quantification or measure of reproducibility was performed in this study, although quantification was applied in a previous study [17]. Owing to the relatively small number of cases, we did not analyse the difference in enhancement pattern by lesion depth, which may have influenced the observed enhancement pattern; however, the influence would have been small because the current high-end machine would have depicted the lesion clearly at such depth. Finally, associated diffuse liver diseases such as fatty liver or cirrhosis would have influenced the liver haemodynamics. However, also because of the small number cases, this influence was not analysed in the current study and further study is mandatory.

In conclusion, the imaging findings of ICC on CEUS were related to the degree of carcinoma cell proliferation at pathological examination. hyperenhancing areas in the tumour always indicated increased density of cancer cells. Understanding the relationship between CEUS enhancement and pathological examination could be helpful in diagnosis, treatment planning and prognostic evaluation for patients with ICC.

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