Review paper

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Diagnosis of intrahepatic cholangiocarcinoma with CEUS

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Abstract

Intrahepatic cholangiocarcinoma (ICC) is a rare, heterogeneous, highly lethal tumor of the biliary tract. Due to the lack of effective treatments, an early identification of ICC is essential to achieve the best outcome in terms of therapy and prognosis aiming for a curative intent. ICC may arise on a normal liver or with an underlying liver disease, making the diagnosis more difficult and complex. Contrast-enhancement ultrasound (CEUS) is an accurate procedure able to detect ICC-specific contrast vascular pattern, and thus facilitating the correlation between radiological and histopathological findings with high specificity and sensitivity. CEUS has been shown to have a high diagnostic potential in the diagnosis of ICC thanks to the possibility of studying in real time the intralesional microcirculation and evaluating the precocity of the enhancement of the lesion during the arterial phase. All these features allow to differentiate the ICC from hepatocarcinoma (HCC) with high sensitivity and specificity. Furthermore, CEUS is a rapid, non-invasive, non-nephrotoxic or non-allergenic tool. The only limitations CEUS may have are related to the disease site and patient characteristics (obesity) and compliance, including the operator's experience. A clinical evaluation of the patient, together with tumor markers and biochemical tests assessment, to differentiate ICC from HCC are highly suggested.

Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer after hepatocarcinoma (HCC); it arises from the epithelial cells of the intrahepatic bile ducts, usually proximal to the second-order branches, at a lower frequency compared to extrahepatic cholangiocarcinoma (ECC)[(1)].
ICC has a poor prognosis, so early identification is very important to achieve the best outcome. Surgical resection represents the gold standard treatment and contributes to increasing the survival rate[(1)].

Different imaging investigations are currently used for ICC diagnosis, as Baseline Ultrasound (US) that represents the first-level procedure. Multidetector Computed Tomography (MDCT) and Magnetic Resonance Imaging (MRI) are second- and third-level diagnostic tools, respectively.

The development of second-generation contrast agents and dedicated software has improved the diagnostic capabilities of US in the detection and characterization of focal liver lesions, allowing the evaluation of intralesional vascularization in real time during all the contrast phases[(2)].

Each contrast phase has its specificity, useful for diagnostic purposes. In particular, the evaluation of vascular enhancement is mainly important during the arterial phase, but the assessment in the portal venous and late phases is equally necessary for a correct diagnosis[3].

Contrast media

Nowadays, there are four US contrast agents available for the study of the liver: SonoVue (BraccoSpa, Milan, Italy; first brought out in 2001) consisting of stabilized gaseous microbubbles (sulfur hexafluoride) of the same size or smaller than red blood cells, with diameters inferior to 7 micrometers, stabilized within a phospholipid membrane; Definity (Lantheus Medical, Billerica, MA, USA; introduced in 2001) consisting of stabilized microbubbles of perflutren in a lipid membrane; Optison (GE Healthcare) containing stabilized microbubbles of human serum albumin and octofluoropropane; Sonazoid (Daiichi-Sankyo, GE Tokyo, Japan, introduced in 2007) consisting of stabilized gaseous microbubbles (perfluorobutane) and a phospholipid membrane (hydrogenated egg phosphatidyl serine)[2]. Definity and Optison have been employed for cardiological imaging only in the USA and Canada. In Canada, Definity is also used for other body areas. Sonozoid is in use just in Japan, while SonoVue is used only in Europe and China. Optison in Europe is approved exclusively for cardiological imaging.

In our article, we will refer exclusively to SonoVue, the only US contrast medium authorized in Europe for the study of focal liver lesions (FLLs)[4].

Basic of CEUS

SonoVue consists of microbubbles of stabilized phospholipids containing sulfur-hexafluoride, with the same dimensions as red blood cells or smaller (i.e. less than seven micrometers). The microbubbles act as a "blood pool agent" and permit the direct study of macro- and microvascular circulation for several minutes[5–7]. This interchange between the microbubble pool and the incident US beam is fundamental to understand the mechanism of the function of the US contrast agent and its clinical uses. When the microbubbles are struck by the US beam at low mechanical index (MI) (<100 kPa – MI <0.1), they are subjected to a low-level positive (compression) and negative (dilation) sound pressure. Consequently, the microbubbles act in a linear way like basic reflectors and do not break. As result, a linear reflection occurrence is created, causing a wide reinforcement of scattering that comes from the circulating blood. This increases the acoustic strength of the incident beam (MI between 0.1 and 1), the oscillation becomes stronger and more asymmetric, and the physical activity of the microbubbles becomes non-linear. Due to the non-linear reflection, if the microbubbles are struck by an acoustic beam with this intensity, a reinforcement of the fundamental signal and harmonic energy will be created. The non-linear microbubble activity can be seen as similar to stationary tissue. The principal advantage of this technique is represented by the quantity of signal that comes from the second harmonic, originating from the microbubbles, which is of greater length than that which comes from stationary tissues. Using dedicated software, linear signals can be removed from the tissues, and images are formed due to the non-linear signals originating from the microbubbles. The use of more intense acoustic waves breaks part of the microbubbles. To reduce the probability of such an occurrence, we selected low mechanical indices, allowing us to delete the signal from the tissues and permitting pure images exclusively from the microbubbles[7–11]. The correct setting of the US scanner and the techniques used for scanning are significant for preventing artifacts[12], MI and inadequate gain are the most relevant causes of error in visualizing signals from the tissues.

Diagnosis of ICC

B-Mode US

B-Mode US typically shows ICC as a solitary lesion, presenting as a homogeneous mass with regular or irregular margins, mainly hypoisoechoic if smaller than 3 cm in diameter, and hyperechoic or mixed if larger than 3 cm in diameter[13]. Necrosis, fibrosis and active growth tumor can cause heterogeneous appearance of the lesion[14]. A peripheral hypoechoic halo representing the compressed hepatic parenchyma may be associated. Another often detectable aspect is the segmental dilation of the corresponding biliary tract and ductal amputation within the tumor area. Larger tumors can infiltrate the hepatic vessels, causing lobar/segmental atrophy, capsular retraction (a specific sign for ICC), and bile duct bundle[13].

Color-Doppler evaluation shows poor intralesional vascularity. It has a high sensitivity in evaluating portal obstruction (100%) and neoplastic involvement (infiltration) of the portal vein (sensitivity 83%, specificity 100%)[15].

However, there are also nodular forms containing mucin, represented on US as well-circumscribed cystic masses. Intralesional calcifications, obstruction of the bile duct distally to the tumor, and occasionally abnormal distension of the bile ducts may occur[16].

CEUS procedures

A B-mode evaluation is mandatory to perform before starting CEUS evaluation; the site, size, and echogenicity of the lesion must be analyzed as well as its relationship with vascular structures.

Color-Doppler is also helpful in the evaluation of the vascular pattern of the lesion (central or peripheral vessels). When the lesion has been recognized, the procedure can start at low MI. A 20 G needle is used for the injection of SonoVue into the antecubital vein and, subsequently, a bolus of 20 ml solution of sodium chloride is administered. The size of the needle should not be less than 20 G, to avoid the destruction of the microbubbles during administration[2]. Real-time examination of the lesion and the surrounding parenchyma is performed for 5–10 minutes. The entire examination is recorded and transferred in a video support. The arterial phase is defined as 0–30 seconds from the start of injection of the contrast medium, the portal phase as 31–75 seconds, and the late phase as 70–180 seconds up to 10 minutes[17].

CEUS findings

The most common finding in ICC is peripheral arterial rim enhancement compared to the surrounding parenchyma (Fig. 1), which appears in 50–73.3% of cases[18].

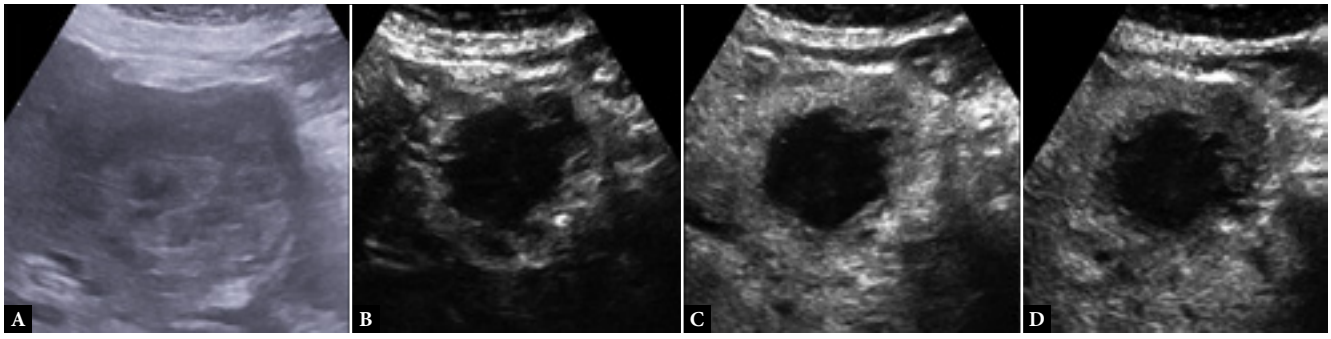

Fig. 1. A. *B-Mode ultrasound shows slightly hyperechogenic, inhomogeneous ecostructure nodule with slightly rough and irregular hypoechoic margins;* **B.** *Arterial phase (20 s): rim-like peripheral hyperenhancement with central microcirculation;* **C.** *Portal phase (70 s): the nodule has peripheral wash-out with central hypoenhancement*; **D.** *Late phase: ICC nodule presenting hypoenhancement*

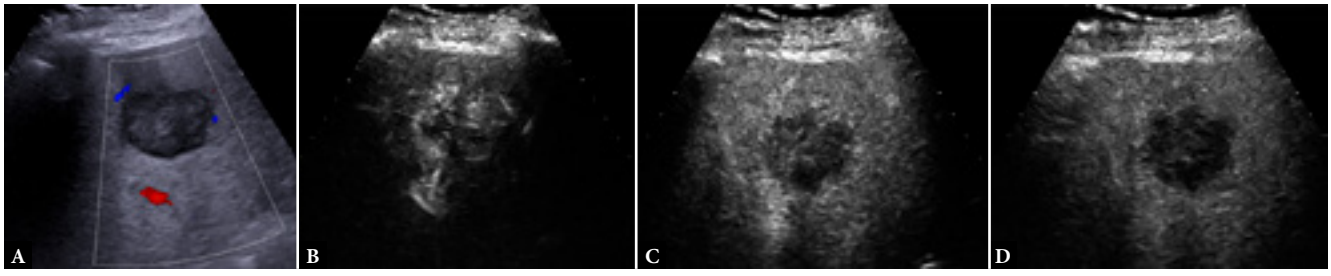

Fig. 2. A. *B-Mode ultrasound shows an unevenly hypoechoic nodule at finely drafted irregular margins, showing no intralesional vascularization at color-Doppler;* **B.** *Arterial phase (22 s);* **C.** *Portal phase (65 s): slight persistence of peripheral enhancement with intralesional contrast agent wash-out; D: Late phase: nodule hypoenhancement*

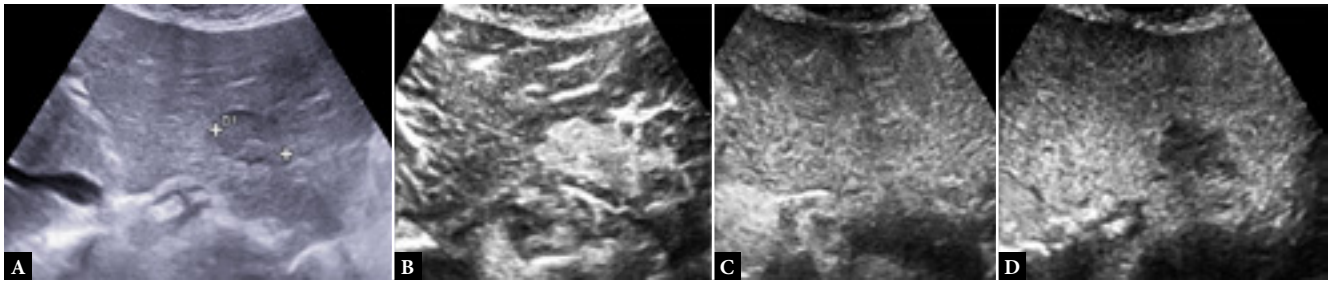

Fig. 3. A. *B-Mode ultrasound shows a homogenous isohypoechoic nodule with thin peripheral hypoechoic capsule;* **B.** *Arterial phase (17 s): complete and homogeneous hyperenhancement of the nodule; nodule presents in portal phase (***C***, 70 s) and late (***D***) hypoenhancement*

Other, relatively rare, findings of the arterial phase include heterogeneous hyperenhancement (Fig. 2), homogeneous hyperenhancement (Fig. 3) or heterogeneous hypoenhancement.

During the portal and late venous phases, the lesion has a hypovascular aspect. However, nodules larger than 5 cm in diameter or arising on a cirrhotic liver present more likely homogeneous hyperenhancement in the arterial phase. Furthermore, the time/intensity curve facilitates differential diagnosis with HCC: the curve during the arterial phase is earlier and lower, with more rapid wash-out during the portal venous (<60 s) and late phases for ICC compared to HCC (Fig. 4).

These findings have a sensitivity of 86.7%, specificity of 95.8%, and accuracy of 90.7% in the differential diagnosis between ICC and HCC in non-cirrhotic livers, increasing the diagnostic effectiveness of CEUS (58.3% sensitivity, 90.0% specificity, and 75.9% accuracy)[19].

The differential diagnosis of ICC also includes metastases, which typically appear hypovascular in all phases. In particular, during the arterial phase they show a peripheral enhancement with soft persistence in the portal phase and wash-out during the late phase[20].

Intraductal ICC shows peripheral capsular contrast-enhancement during the arterial phase, homogeneous and complete contrast-enhancement during the venous phase, and wash-out during the late phase (Fig. 5).

In a more recent literature review, the sensitivity, specificity and overall diagnostic accuracy values confirm high diagnostic confidence in ICC diagnosis, as reported in Tab. 1. The same data applied to CT and MRI are reported in Tab. 2.

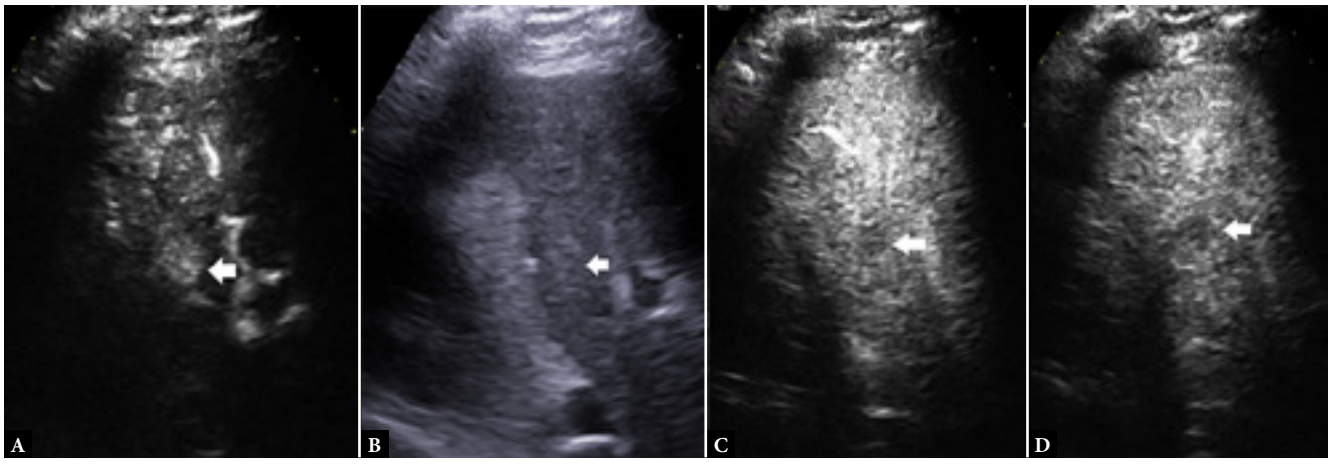

Fig. 4. *CEUS in a typical HCC.* **A.** *B-Mode ultrasound shows an isohyperechoic nodule (arrow);* **B.** *Arterial phase (30 s): complete and homogeneous hyperenhancement of the nodule (arrow);* **C.** *In the portal phase (90 s), the nodule appears slightly hypovascular (arrow);* **D.** *In the late phase (180 s), the nodule appears completely hypovascular (arrow)*

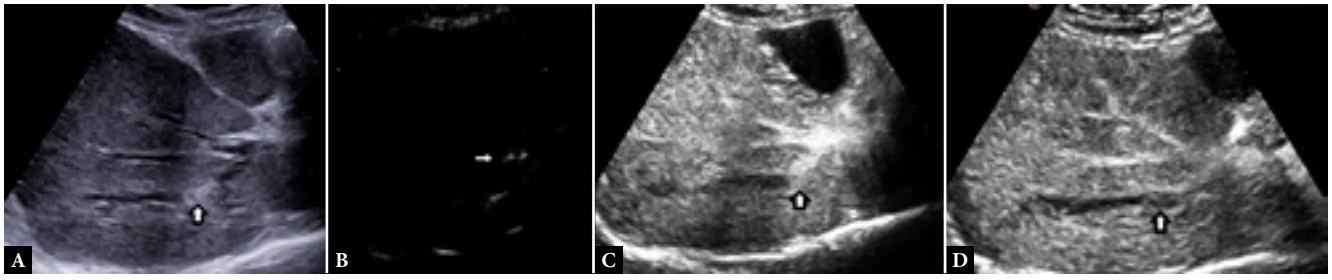

Fig. 5. A. *B-Mode ultrasound shows a circumscribed isodense polypoid overhang in the context of the biliary intrahepatic pathway at the seventh segment (arrow);* **B.** *In the arterial phase, there is a partial capsular peripheral enhancement (arrow);* C. *In the portal phase, the intraductal ICC nodule presents tenuous hyperenhancement (arrow);* **D.** *In the late phase, the nodule presents hypoenhancement (arrow)*

Discussion

Second-generation contrast agents and dedicated software allow a better evaluation of real-time perfusion, improving the study of small lesions.

In particular, a contrast agent based on microbubbles with sulfur hexafluoride has wide applications in the diagnosis of focal liver lesions, as it is distributed at the endovascular level, without any passage into the interstitial space. Furthermore, it permits the analysis of the entire hepatic parenchyma. In turn, contrast agents used in CT and MRI assessments can pass through the interstitium of the lesion, resulting in long-lasting enhancement. Considering these features, CEUS can display intrahepatic vascular characteristics more clearly, giving additional information during the late phase[21].

Following contrast agent administration, four different enhancement patterns are reported in the arterial phase (<30 seconds): irregular peripheral hyperenhancement, heterogeneous hyperenhancement, homogeneous hyperenhancement, and heterogeneous hypoenhancement. In the portal venous phase (30–120 seconds), the lesion shows hypoenhancement. During the late phase (>120 seconds), the lesion shows hypoenhancement. In particular, according to Chen *et al.*[22], the portal-venous peripheral enhancement rim (present in 66.7% of the lesions under evaluation in their retrospective study) can confirm the diagnosis of ICC (conversely, infrequent in HCC) as an expression of infiltrative tumor growth[22].

The enhancement pattern of ICC in the arterial phase can be affected by cirrhosis or concomitant viral hepatitis[23] or by the size of the tumor. In particular, ICCs with dimension less than 5 cm generally show homogeneous enhancement (due to its minor fibrotic

Tab. 1. Sensitivity, specificity, diagnostic accuracy, and positive and negative predictive values in the diagnosis of ICC by CEUS. Review of recent literature

Author	Sensitivity	Specificity	Diagnostic accuracy	PPV	NPV
Vidili *et al.* (2022)[48]	91.3%	96.7%	96.5%	56.8%	99.6%
Dong *et al.* (2023)[19]	86.7%	95.8%	90.7%	–	–
Ainora *et al.* (2023)[18]	80%	83.8%	–	82.3%	81.6%
Li *et al.* (2014)[40]	78.8%	88%	84.3%	81.3%	86.3%

Tab. 2. Sensitivity, specificity, diagnostic accuracy, and positive and negative predictive values in the diagnosis of ICC by CT and MRI. Review of recent literature

Author	Sensitivity	Specificity	Diagnostic accuracy	PPV	NPV
Li *et al.* (2022)[49]	CT: 79.4% MRI: 84.1%	CT: 67.1% MRI: 65.7%	–	–	–
Lee *et al.* (2010)[50]	CT: 84.2%	CT: 70.6%	CT: 81.8%	CT: 93.2%	CT: 48%
Kim *et al.* (2021)[51]	CT: 83.4% MRI: 83.4%	CT: 96.4% MRI: 97.6%	CT: 89.8% MRI: 90.4%	–	–
Nisioka *et al.* (2022)[52]	CT: 49% MRI: 51%	CT: 100% MRI: 97%	CT: 80% MRI: 79%	CT: 100% MRI: 92%	CT: 75% MRI: 76%
Petrowsky *et al.* (2006)[53]	CT: 78%	CT: 80%	CT: 79%	CT: 92%	CT: 57%
Ke *et al.* (2023)[54]	CT: 60.98% MRI: 95.12%	CT: 76.92% MRI: 96.15%	CT: 69.89% MRI: 95.7%	–	–

component), but if their diameter is greater than 5 cm, they typically present peripheral enhancement in the arterial phase and hypovascularization in the portal and late phases.

Jin et al. retrospectively analyzed a total of 64 ICCs, divided into two groups depending on tumor size, including 25 with dimensions <5 cm and 39 >5 cm. In the former group, at the baseline 84% had a hypoechoic echostructure and 16% were heterogeneous; in the latter group, at the baseline 5.1% had isoechoic, 76.9% had hypoechoic, and 17.9% had heterogeneous reactions. During the arterial phase, in the former group 31.3% had homogeneous hyperenhancement, 17.2% partial hyperenhancement, and 51.6% peripheral enhancement; in the latter group, 64% had homogeneous hyperenhancement, 8% partial hyperenhancement, and 28% peripheral enhancement. During the portal venous phase, in the former group 16% had an isoechogenic aspect and 84% a hypoechoic aspect; in the latter group 7.7% had an isoechoic aspect and 92.3% a hypovascular aspect. In both groups, 100% of lesions were hypovascular in the late phase[24].

On the other hand, in presence of cirrhosis, homogeneous hyperenhancement in the arterial phase (64% of the cases in the first group) can be often detected[24], probably in relation to the development of arterial neovascularization and small vessels in this pathological condition[25].

The grade of microvascular density, arterial density, fibrous stroma, and necrosis may be responsible for the differences in the aforementioned enhancement patterns[12,23].

In the clinical practice, CT findings are considered conclusive for the diagnosis in almost all patients, while MRI is only used in specific patients[26].

In addition, hepatospecific contrast agent (BOPTA and/or EOB) MRI may allow later sequences (cholangiography) to demonstrate hepatocyte alteration characterized by low signal intensity in the lesion, providing additional diagnostic evidence of malignancy[27].

Xu et al. retrospectively analyzed histologically CEUS patterns of 40 ICCs, with semi-quantitative assessment of tumor cell grade and of the distribution of endolesional fibrosis, demonstrating that CEUS findings correlate with the degree of proliferation of cancer cells seen during histopathological examination. Areas of endo tumor hyperenhancement, in particular, always indicate an increase in tumor cell density. In this study, 68.8% of the lesions were hypoechoic, 15.6% isoechoic, and 15.6% hyperechoic. In the arterial phase, 59.4% had irregular peripheral hyperenhancement, 18.8% heterogeneous hyperenhancement, 9.4% homogeneous hyperenhancement, 12.5% homogeneous hypoenhancement, and 28.1% had endolesional arterial vessels. In the portal phase, 96.9% had hypoenhancement, and 3.1% isoenhancement. In the late phase, 100% of lesions had hypovascular appearance[28].

CEUS is also well-recognized for its ability to characterize liver lesions; its excellent diagnostic value was confirmed by several prospective studies, including the DEGUM study with over 1,000 patients[29], the Romanian study[30] and the French multicenter study[31].

CEUS has been shown to characterize liver tumors with the same accuracy as CT and MRI[32,33]. Therefore, CEUS was included among the imaging techniques approved for the non-invasive diagnosis of HCC in the 2005 American Association of the Study of Liver Disease (AASLD) guidelines[34]. However, in a retrospective series of histologically confirmed cirrhosis patients with ICC, the Barcelona Clinic Liver Cancer (BCLC) team found out ten ICC patients with CEUS enhancement pattern similar to those considered diagnostic for HCC, or homogenous arterial enhancement followed by washout[35].

Based on these findings, CEUS was removed from the diagnostic techniques for nodules in cirrhosis in the AASLD 2011 guidelines[36] and subsequently also from the European Association for the Study of the Liver (EASL) guidelines[37,38]. However, this removal has raised many controversial questions and has not been well received in Europe and Asia[39].

In fact, Li et al. analyzed and compared the contrast kinetics of 33 ICCs and 50 HCCs in cirrhotic livers, demonstrating that CEUS is useful to discriminate between these two types of lesions. In particular, if a nodule in a cirrhotic liver shows hyperenhancement in the arterial phase, followed by early and marked portal wash-out (within 60 seconds), the nodule is strongly suspected for ICC rather than HCC. In the HCC, in fact, the wash-out is less marked and slower (>60 seconds). Considering early washout and the portal phase as diagnostic criteria, CEUS has a sensitivity of 78.8%, a specificity of 88%, a positive predictive value of 81.3%, a negative predictive value of 86.3%, and an accuracy of 84.3%[40].

Tumor markers represent an additional diagnostic tool for the diagnosis of ICC and for the differential diagnosis with HCC. In particu-

lar, CA19-9 shows increased values in patients with ICC[41], while alpha-fetoprotein (AFP) in HCC[42].

Hu et al. used CEUS to evaluate 50 indeterminate nodular lesions on MRI, exploring in particular the detection and characterization of the wash-out, and concluding that CEUS is complementary to MRI and can be used to characterize indeterminate liver lesions on MRI[43].

CEUS can be used in patients undergoing radiofrequency therapy (RFA) when the target is not clearly evident at baseline US. Also, it can be performed during treatment with RFA to detect the presence of microvascularization within the lesion, making possible to evaluate therapeutic effectiveness during the monitoring of patients undergoing percutaneous (RFA) and transarterial treatments[44]. Comparing the available literature data, RFA demonstrates a similar overall survival rate in the treatment of patients with ICC and HCC. In particular, the three-year survival rate is 65% in ICC[45] and 59.7% in HCC[46].

The intrinsic limitations of CEUS can vary in relation to various patient characteristics (obesity, compliance), injury (localization, size, depth), and operator confidence[47].

Another important limitation of CEUS consists of the examination performed on a single target, mainly in the arterial phase, while it can be particularly difficult to evaluate the entire hepatic parenchyma in a short period of time. In contrast, CT and MRI make it possible to evaluate the entire hepatic parenchyma.

When a differential diagnosis between benign and malignant lesions is not possible despite the use of different imaging methods, a strict follow-up of the patient or a biopsy could be necessary, depending on the size of the lesion and clinical considerations.

Conclusions

CEUS is a non-invasive, stress-free, rapid, cost-efficient, and accurate tool for the diagnosis and management of ICC; CEUS can be easily repeated and avoids radiation exposure. CEUS is not nephrotoxic or allergenic, and allows rapid characterization with good precision when carried out by experienced physicians.

Thanks to the development of dedicated software, CEUS has been shown to have a high diagnostic potential in the diagnosis of ICC, allowing the possibility of studying in real time the intralesional microcirculation and evaluating the precocity of wash-in and wash-out of SonoVue during the arterial phase. All these features help to differentiate ICC from HCC with high levels of sensitivity and specificity.

Conflicts of interest

The authors do not report any financial or personal connections with other persons or organizations which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Author contributions

Original concept of study: GGV, FL. Writing of manuscript: GGV, FL. Analysis and interpretation of data: GGV, ML, AT, RM, GC, LM, IVT, MM, AP, AC, GL, DG, MT, FF, PF, FL. Final approval of manuscript: GGV, ML, AT, RM, GC, LM, IVT, MM, AP, AC, GL, PF, FL. Collection, recording and/or compilation of data: GGV, ML, AT, RM, GC, LM, IVT, MM, AP, AC, GL, SL, SvL, DG, MT, FF, FL. Critical review of manuscript: GGV, ML, AT, RM, GC, LM, IVT, MM, AP, AC, GL, DG, MT, FF, PF, FL.

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