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● *Original Contribution*

CONTRAST-ENHANCED ULTRASOUND PATTERNS FOR THE NON-INVASIVE DIAGNOSIS OF HEPATOCELLULAR CARCINOMA: A PROSPECTIVE MULTICENTER STUDY IN HISTOLOGICALLY PROVEN LIVER LESIONS IN A REAL-LIFE SETTING DEMONSTRATING THE BENEFIT OF EXTENDED LATE PHASE OBSERVATION

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Abstract—The hallmark for the non-invasive diagnosis of hepatocellular carcinoma (HCC) with contrast-enhanced ultrasound (CEUS) in cirrhosis is arterial phase hyperenhancement (APHE), followed by late-onset (>60 s), mild washout. Large retrospective studies report this pattern of washout to occur in the vast majority of HCCs. However, a prospective multicenter validation of these findings is still missing. Thus, we initiated a prospective multicenter validation study assessing CEUS enhancement patterns in focal liver lesions of patients at risk for HCC. We analyzed lesions that were eventually histology proven in a real-life setting. CEUS patterns were assessed for subgroups of HCC, intrahepatic cholangiocellular carcinoma (iCCA) and non-HCC, non-iCCA lesions. The diagnosis was HCC in 316 lesions (median size: 40 mm), iCCA in 26 lesions (median size: 47.5 mm) and non-HCC, non-iCCA in 53 lesions (median size: 27 mm). Overall, 85.8% of HCCs exhibited APHE. APHE followed by washout occurred in 72.8% of HCCs and 50% of iCCAs and non-HCC, non-iCCA malignancies ($p < 0.05$). Early and marked washout was associated more commonly with iCCA; HCCs exhibited mostly late and mild washout (onset >4–6 min in 10% of cases). Our prospective data confirm that the typical pattern of APHE followed by late-onset, mild washout occurs in the majority of HCCs. (E-mail: barbara.schellhaas@uk-erlangen.de)

Key Words: **Hepatocellular carcinoma, Intrahepatic cholangiocellular carcinoma, Contrast-enhanced ultrasound, Cirrhosis, Non-invasive diagnosis, Imaging, Ultrasound, Guidelines, Contrast enhancement pattern, Examination standard.**

INTRODUCTION

Hepatocellular carcinoma (HCC) occurs mainly in the cirrhotic liver and can be diagnosed non-invasively with contrast-enhanced imaging in high-risk patients. The characteristic enhancement pattern of HCC is defined as arterial phase hyperenhancement (APHE), followed by contrast washout in the portal venous or late phase (hyper–hypo pattern). More recent versions of HCC guidelines suggest a more refined definition of the typical enhancement pattern as APHE followed by mild and late-onset (>60 s) washout in nodules ≥10 mm in cirrhosis, with, however, the exact wording differing between various guidelines (Greten et al. 2013; American College of Radiology (ACR) 2021; Kono et al. 2017; Galle et al. 2018) (Supplementary Table S1, online only). Large retrospective studies suggest this typical washout pattern occurs in the vast majority of HCCs (Terzi et al. 2018); however, these findings have not been validated in a prospective multicenter real-life setting yet. Moreover, the rate of HCCs with a very late onset of washout has been poorly investigated so far.

In addition, although there are recommendations in the literature that a late examination point in the late

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phase (after >4−6 min) seems sensible, particularly in HCC (Claudon et al. 2013; Schellhaas and Strobel 2019), the late phase for contrast-enhanced ultrasound (CEUS) examination procedures is defined as ">2 min," with the recommendation of a longer late phase >4 min in cirrhotic liver in updated WFUMB guidelines (Dietrich et al. 2020). Thus, the habit of ending the late phase after 2−3 min might result in overlooking the typical CEUS pattern in those HCCs with very late onset of washout. Also, despite increasing evidence in the literature that especially well-differentiated HCCs exhibit very late, mild washout or even no washout at all (Boozari et al. 2011; Leoni et al. 2013; Giorgio et al. 2016; Schellhaas et al. 2016), arterial phase hyperenhancement alone (hyper−iso pattern) in CEUS is not yet considered sufficient for the definite diagnosis of HCC in cirrhotic liver (Galle et al. 2018).

The German Society for Ultrasound in Medicine (DEGUM) CEUS HCC study has been described in detail elsewhere (Schellhaas et al. 2021; Strobel et al., 2021). The study was registered as a National Institutes of Health (NIH) trial (NCT03405909) and funded by DEGUM. Briefly, this prospective multicenter study intended to assess the diagnostic accuracy of standardized CEUS for the non-invasive diagnosis of HCC in high-risk patients in a real-life setting.

This article represents a subanalysis of the prevalence and diagnostic accuracy of different CEUS patterns for the non-invasive diagnosis of HCC using histology as the gold standard. CEUS examinations were standardized, and included an additional examination point in the late phase after 4−6 min in lesions with no contrast washout at 3 min to assess the diagnostic impact of the onset and intensity of washout in lesions with a hyper−hypo pattern. The major issue to assess was the prevalence and distribution of CEUS patterns in HCC compared with intrahepatic cholangiocellular carcinoma (iCCA) and non-HCC, non-iCCA lesions, with a focus on the presence on APHE with and without washout as well as the onset and intensity of washout. The study was intended to validate the findings of the typical HCC pattern (APHE followed by late-onset, mild washout) from large retrospective studies in a prospective multicenter real-life setting in histology-proven lesions.

METHODS

Study design

The study design is illustrated in Figure 1. Patients at high risk for HCC according to national HCC guidelines with a solid liver lesion visible on B-mode ultrasound were recruited prospectively to undergo CEUS followed

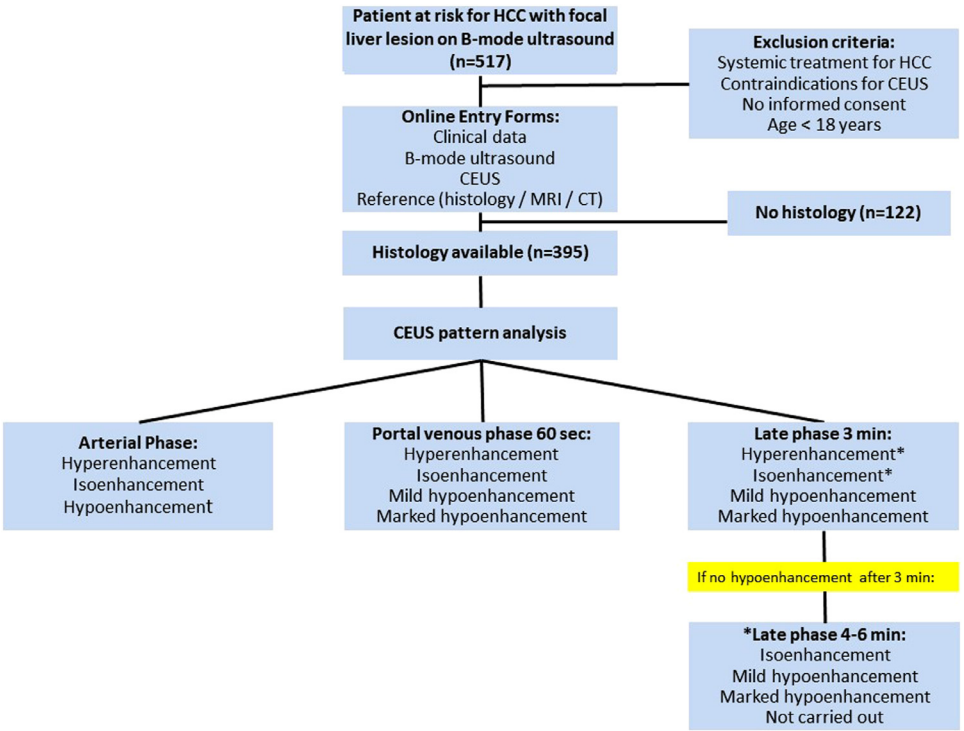

Fig. 1. Study design. CEUS = contrast-enhanced ultrasound; CT = computed tomography; HCC = hepatocellular carcinoma; MRI = magnetic resonance imaging.

by further diagnostic workup. Exclusion criteria were previous systemic or locoregional treatment for HCC, age <18 y, the absence of histology as reference standard, contraindications to ultrasound contrast agents and lack of informed consent. In the case of patients with more than one focal liver lesion, only the best accessible one was chosen as an index lesion for CEUS and further assessment. Clinical and imaging data (B-mode ultrasound, CEUS) were entered into password-protected online forms (Schellhaas et al. 2021). The following items were recorded: patient age, gender, risk factor, presence of diabetes mellitus, history of extrahepatic malignancy, patient's general condition according to Eastern Cooperative Oncology Group (ECOG) performance status, presence of liver cirrhosis and stage according to the Child–Pugh classification, B-mode findings (status of liver parenchyma, presence of portal vein thrombosis on B-mode ultrasound/color mode, transjugular intrahepatic portosystemic stent shunt [TIPSS], number of focal liver lesions); index lesion (size, echogenicity, presence of hypo-echoic rim, macro-invasion of the liver vessels, depth location); CEUS findings (enhancement behavior of the index lesion in relation to the surrounding liver parenchyma in the arterial phase, portal venous phase at 1 min, late phase at 3 min and, optionally, at 4–6 min); application of a second contrast bolus; presence of enhancing tumor thrombus; optionally, categorization of the index lesion according to the standardized CEUS algorithms ESCULAP and CEUS LI-RADS; histological findings from the index lesion and liver parenchyma. The local ethics committee approved the study (Ethics Vote 16_17B). All patients provided their written informed consent according to DSGVO 05/2018 (European General Data Protection Regulation) for prospective evaluation of anonymized data.

Standardized contrast-enhanced ultrasound

All ultrasound examinations were performed using high-end ultrasound devices (Siemens Acuson S2000, Siemens Acuson S3000, Siemens Acuson Sequoia, Siemens Medical Solutions, Erlangen, Germany; Supersonic Aixplorer, SuperSonic Imagine, Aix-en-Provence, France; Hitachi Preirus, Hitachi 6500 HV, Hitachi Ascendus, Hitachi Arietta 850, Hitachi Medical Systems, Wiesbaden, Germany; GE Logiq E9, GE Logiq S8, GE Healthcare Co., Solingen, Germany; Toshiba Aplio 500, Toshiba i800, Toshiba Aplio XG, Canon Medical Systems, Neuss, Germany; Philips Epiq 5, Philips IU22, Philips GmbH Market DACH, Hamburg, Germany; Samsung RS 85, Samsung Electronics GmbH, Schwalbach, Germany).

Contrast-enhanced ultrasound was performed using a standardized protocol with continuous assessment of the arterial phase until maximum contrast enhancement was reached in the lesion, followed by intermittent scanning with short sweeps through the lesion at the following time points: 1 min, 3 min and 4–6 min for cases of no contrast washout after 3 min (Fig. 2) (Schellhaas and Strobel 2019). In the case of insufficient contrast enhancement in the late phase, examiners were instructed to apply a second contrast bolus with subsequent assessment of the late phase only. This approach was chosen to avoid insufficient enhancement caused by the unintended disruption of microbubbles through too much scanning in the arterial or portal venous phase, which might mimic hypo-enhancement. In case of

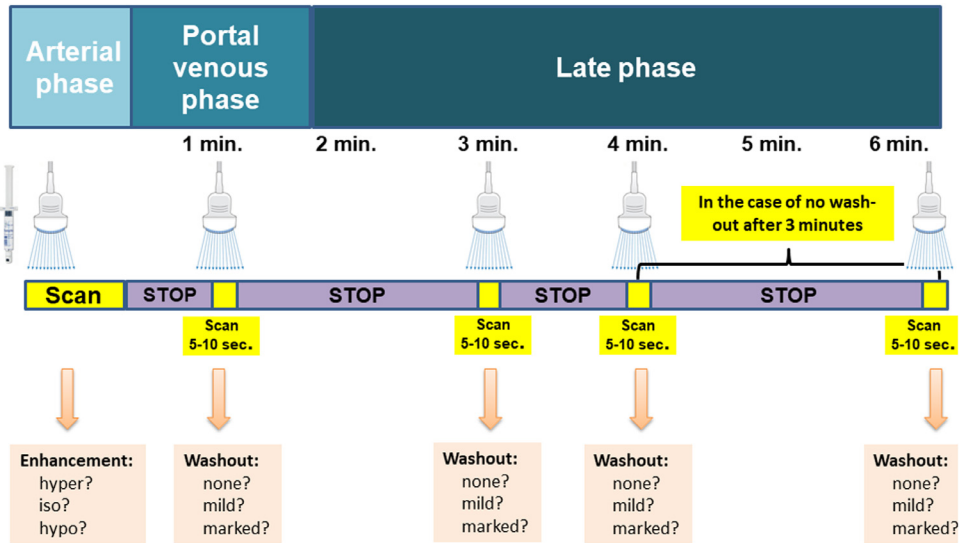

Fig. 2. Contrast-enhanced ultrasound (CEUS) examination standard.

contrast washout, examiners had to classify the extent of washout as either mild or marked by means of their subjective impression at 60 s, 3 min and, in case of no washout at 3 min, 4−6 min.

Statistical analysis

Data were exported from the online entry forms using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA). Quantitative variables were expressed as the mean and range. Categorical variables were expressed as frequencies. Sensitivities, specificities and positive and negative predictive values were calculated. The data were tested for a normal distribution using the Kolmogorov−Smirnov test. Groups were compared using a two-sample Student *t*-test for quantitative variables and Fisher's exact test for categorical variables. SPSS-21 (IBM Corp., Armonk, NY, USA) and Excel 2010 (Microsoft Corp.) were used for statistical analyses. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Patient and tumor characteristics

In total, 395 patients (male/female = 329/66, mean age: 67 y, range: 29−88 y) at high risk of HCC with histology-proven lesions were analyzed. Risk factors according to patients' histories were liver cirrhosis, n = 302 (76.5%); chronic hepatitis B infection, n = 19 (4.8%); chronic hepatitis C infection with advanced fibrosis, n = 21 (5.3%); non-alcoholic steatohepatitis (NASH), n = 19 (4.8%); history of prior HCC on the background of glycogenosis, n = 1 (0.3%). In the remaining cases, more than one risk factor was present (Supplementary Table S2, online only).

Tumor diagnoses based on histology were hepatocellular carcinoma (HCC), n= 316; intrahepatic cholangiocellular carcinoma (iCCA), n = 26; and other malignancy, n = 19 (metastases, n = 13; mixed tumor HCC/iCCA, n = 3; angiosarcoma, n = 3). Thirty-four lesions (8.6%) were benign, with 20 regenerate/dysplastic nodules, 2 cases of focal nodular hyperplasia, one hemangioma, four adenomas and seven other lesions (inflammatory pseudo-tumor in a patient with Erdheim−Chester syndrome, n = 1; "necrosis in vasculitis," n = 1; liver abscess, n = 1; "focal fat/fibrosis," n = 1; inconclusive histology with re-biopsy recommended, n = 3).

Lesion characteristics

The mean size of the index lesion was 57.6 mm (range: 5−200 mm). Forty-two lesions (10.6%) were <2 cm in size, among these were 28 HCCs. One hundred thirty-six lesions were <3 cm in size (34.4%); 120 were 3−5 cm (30.4%); and the remaining 139 lesions were >5 cm (35.2%). Median size was 40 mm for HCC, 47.5 mm for iCCA and 27 mm for non-HCC, non-iCCA lesions. Macro-invasion of the portal vein or liver veins was seen in 47 lesions (11.9%). Forty-one patients (10.4%) had portal vein thrombosis, while 12 (3%) had a TIPSS. In total, 242 lesions were solitary (61.3%); 79 patients (20%) had two to three lesions, 40 (10.1%) had more than three lesions and 34 (8.6%) had diffuse tumor infiltration.

CEUS patterns

Table 1 outlines CEUS patterns in histologically proven HCCs, iCCAs and non-HCC, non-iCCA lesions for direct comparison. Figures 2 and 3 provide details on the onset and intensity of washout in all lesions exhibiting a hyper−hypo pattern.

CEUS patterns in HCC. Briefly, APHE was seen in 271 of 316 HCCs (85.8%), followed by a contrast washout (hyper−hypo pattern) in 230 of 271 cases (84.5%). APHE without contrast washout (hyper−iso pattern) occurred in 41 HCCs (13%); 6 of these HCCs were <2 cm in size. Twenty-seven HCCs (8.5%) exhibited arterial iso-enhancement followed by contrast washout (early washout ≤60 s in 5 cases, marked washout in 2 cases). In 13 HCCs (4.1%), hypo-enhancement was

Table 1. CEUS patterns in HCC, iCCA and non-HCC, non-iCCA lesions

	HCC (n = 316)	iCCA (n = 26)	non-HCC, non-iCCA (n = 53)	
			Malignant (n=19)	Benign (n = 34)
Arterial phase				
Hyperenhancement	271 (85.8%)	14 (53.8%)	11 (57.9%)	17 (50%)
Iso-enhancement	32 (10.1%)	6 (23.1%)	4 (21.1%)	8 (23.5%)
Hypo-enhancement	13 (4.1%)	6 (23.1%)	4 (21.1%)	9 (26.5%)
Hyper−iso pattern (APHE + no washout)	41 (13%)	1 (3.8%)	1 (5.3%)	5 (14.7%)
Hyper−hypo pattern (APHE + washout)	230 (72.8%)	13 (50%)	10 (52.6%)	12 (35.3%)

CEUS = contrast-enhanced ultrasound; HCC = hepatocellular carcinoma; iCCA = intrahepatic cholangiocellular carcinoma; APHE = arterial phase hyperenhancement.

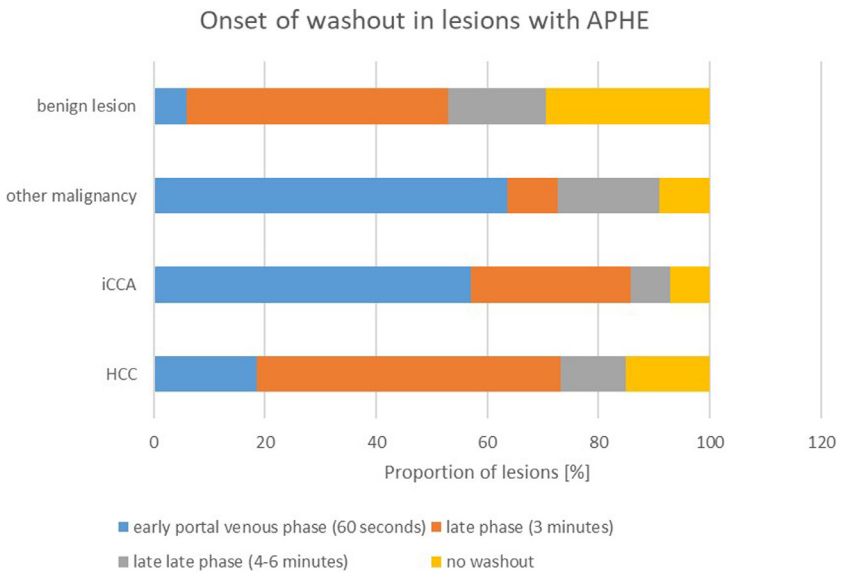

Fig. 3. Onset of washout in lesions with APHE. The size of the colored columns represents the relative proportion of lesions with onset of washout at the given time point, with the time points encoded by the different colors (portal venous phase at 1 min, *blue*; late phase at 3 min, *orange*; late phase after >4−6 min, *gray*; no washout at all, *yellow*). Every column represents a lesion entity (benign lesion, n = 17; other malignancy, n = 11; iCCA, n= 14; HCC, n=271). APHE = arterial phase hyperenhancement; HCC = hepatocellular carcinoma; iCCA = intrahepatic cholangiocellular carcinoma.

seen in the arterial phase. Five of these HCCs exhibited subsequent iso-enhancement in the portal venous and late phases, while 6 exhibited further contrast washout.

In those HCCs with APHE followed by contrast washout, the onset of washout occurred most often in the late phase at 3 min (148/230, 64.3%), but 50 HCCs (21.7%) exhibited early washout in the portal venous phase (Fig. 3). In 32 HCCs, washout was not seen until the additional examination point in the late phase after 4−6 minutes; 5 of these HCCs were ≤2 cm.

The intensity of contrast washout was described as mild in 175 cases (76.1%) and as marked in 55 cases (23.9%; Fig. 4). In 27 HCCs with early washout in the portal venous phase at 60 s, progressive washout (from mild in the portal venous phase to marked in the late phase at 3 min) was perceived. In 32 patients (10.1%), the additional examination point after 4−6 min yielded diagnostic benefit. In 9 patients with sustained iso-enhancement at 3 min, the examination protocol was violated, and the additional examination point after 4−6 min was omitted by the examiner. The typical HCC pattern of APHE followed by late-onset (>60 s) and mild washout occurred in 158 HCCs (50%). An example of typical CEUS findings in HCC is illustrated in Figure 5.

CEUS in iCCA. Overall, 14 of 26 iCCAs (53.8%) exhibited non-rim-like APHE. In 13 of these lesions, contrast washout occurred (hyper−hypo pattern); there was only one case of a hyper−iso pattern. CEUS patterns of iCCAs with APHE are illustrated in Figures 3 and 4.

Six iCCAs (23.1%) exhibited iso-enhancement in the arterial phase; in all but one iCCA, this was followed by contrast washout at some point. In 6 iCCAs, hypo-enhancement was seen in the arterial phase. In one case, this was followed by iso-enhancement in the portal venous phase; 4 cases exhibited further contrast washout. Thus, leaving aside the lesions with primary hypo-enhancement in the arterial phase, all but 2 iCCAs exhibited contrast washout (18/20 = 90%). Early washout in the portal venous phase at 60 s occurred in 12 of the 26 iCCAs (46.2%). The onset of washout was observed in the late phase at 3 min in 5 cases (19.2%), and very late washout beginning after 4−6 min occurred in 2 iCCAs (7.7%; Fig. 3). The intensity of washout was perceived as mild in 9 cases (34.6%) and marked in 10 cases (38.5%; Fig. 4).

CEUS patterns in non-HCC, non-iCCA lesions. Arterial phase hyperenhancement occurred in 50% of the benign lesions (17/34) and 57.9% of the non-HCC, non-iCCA malignancies (11/19). Iso-enhancement and hypo-enhancement in the arterial phase occurred with similar frequencies of around 21%−26% in both benign and malignant non-HCC, non-iCCA lesions.

The hyper−iso pattern was seen in 5 of 34 benign lesions (14.7%): regenerative nodules, n = 4, and a hemangioma, n = 1. Only one non-HCC, non-iCCA

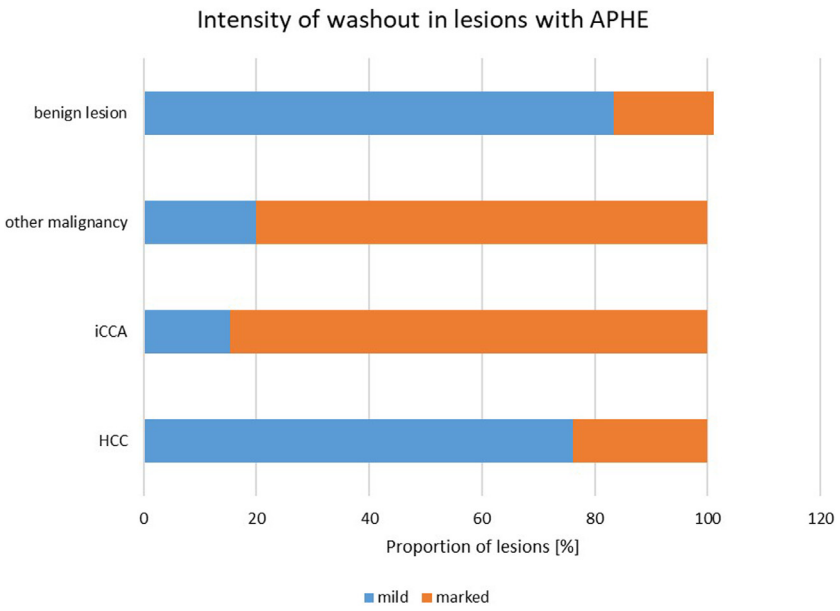

Fig. 4. Intensity of washout in lesions with APHE. The size of the colored columns represents the relative proportion of lesions with a given intensity of washout according to the subjective impression of the examiner), with the intensity encoded by the different colors (blue for mild washout, orange for marked washout). Every column represents a lesion entity (benign lesion, n = 17; other malignancy, n = 11; iCCA, n= 14; HCC, n=271). APHE = arterial phase hyperenhancement; HCC = hepatocellular carcinoma; iCCA = intrahepatic cholangiocellular carcinoma.

malignancy had a hyper−iso pattern; this was a mixed tumor (HCC−iCCA).

The hyper−hypo pattern was also seen in 12 of 34 benign lesions (35.3%). These benign lesions were regenerative nodules, n = 5; focal nodular hyperplasia, n = 2; adenomas, n = 4; and necrosis in vasculitis, n = 1. In the last case, arterial phase hyperenhancement was followed by early and marked washout. In the benign

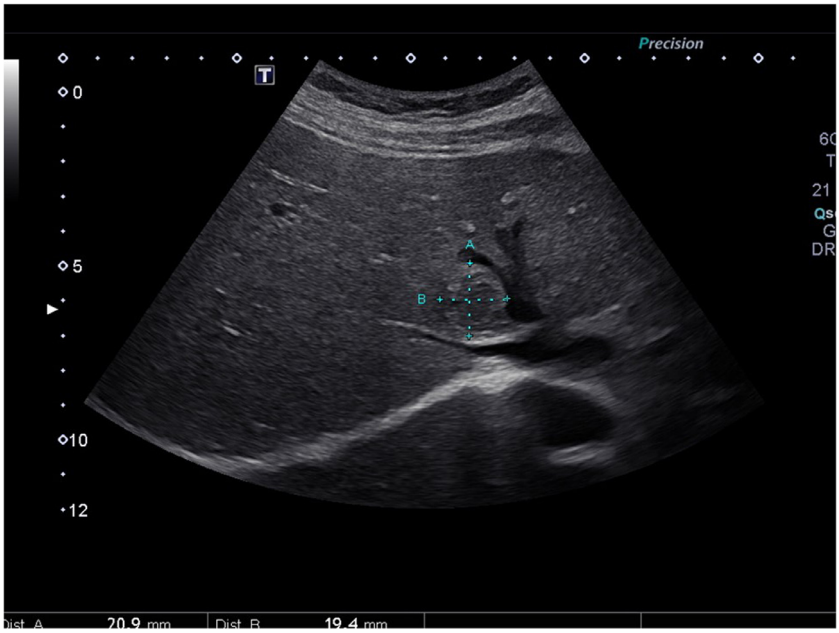

Fig. 5. Typical contrast-enhanced ultrasound images of hepatocellular carcinoma. The lesion shown was an incidental finding in a patient with cirrhosis. (a) B-Mode: slightly hypo-echoic lesion 21 mm in size in segment IV. (b) Color mode: no hypervascularization detectable. (c−f) Contrast-enhanced ultrasound. (c) Arterial phase hyperenhancement. (d) Iso-enhancement in the portal venous phase. (e) Iso-enhancement in the late phase after 3 min. (f) Slight hypoenhancement in the late phase after >4 min. Histology: Hepatocellular carcinoma grade 2 (moderately differentiated).

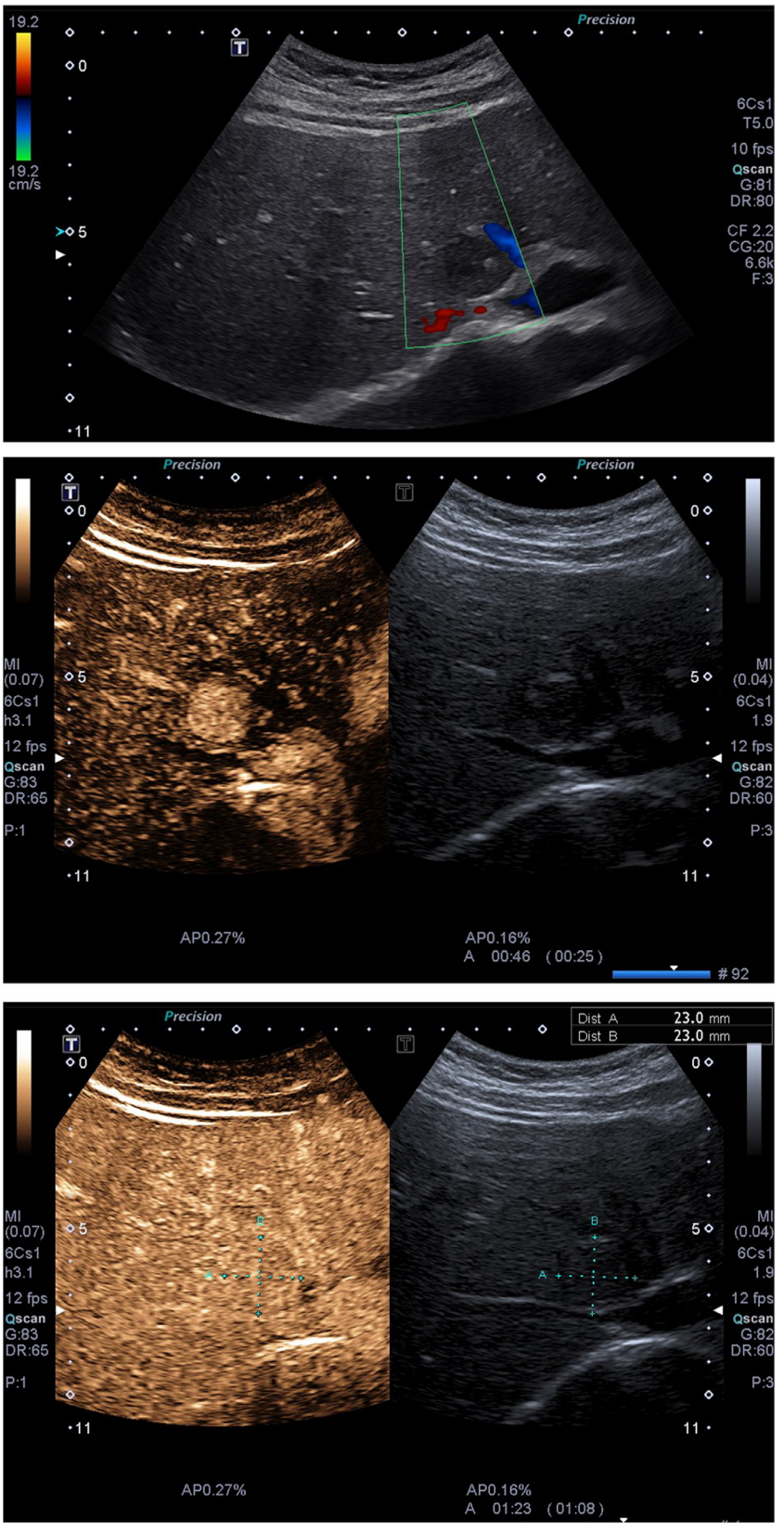

Fig. 5. Continued

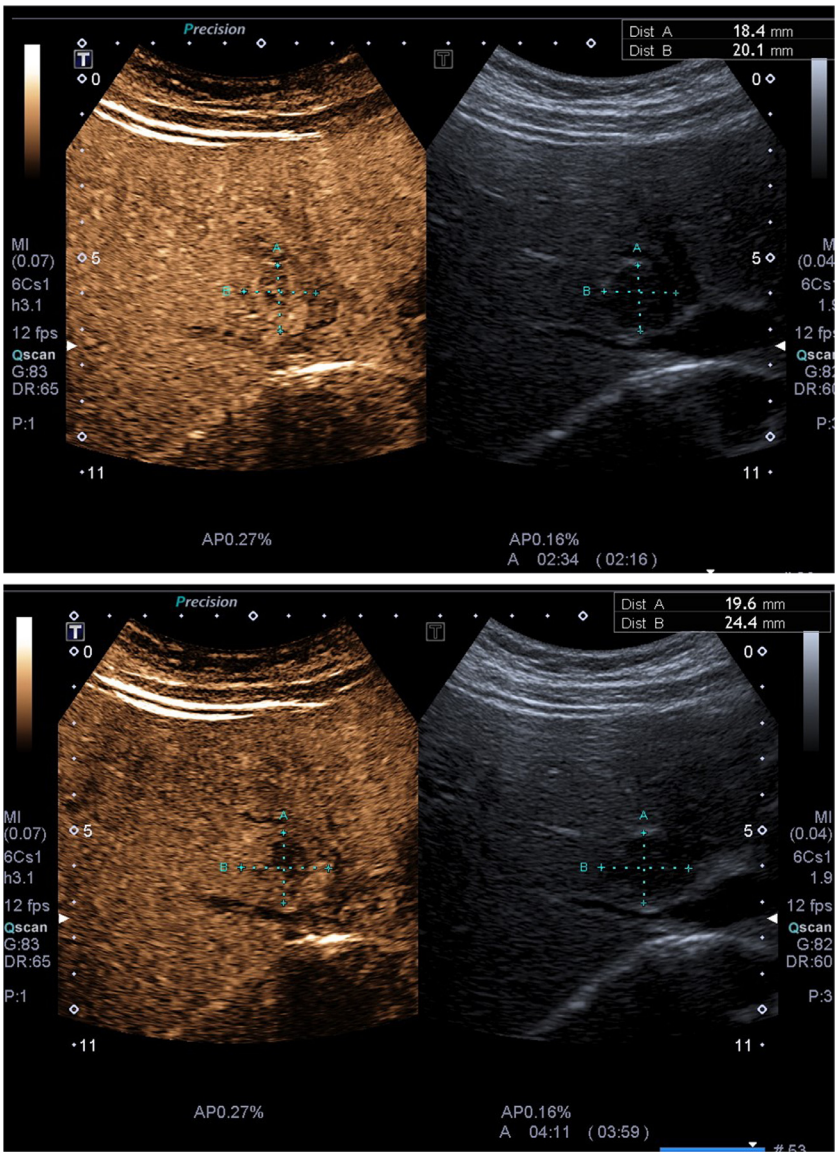

Fig. 5. Continued

lesions, washout tended to occur most often in the late phase (66.7%) or very late phase (25%), whereas malignant lesions most often exhibited early washout in the portal venous phase (70%; Fig. 3).

Concerning the intensity of washout, mild washout was associated with benign lesions, whereas malignant lesions most often exhibited marked washout (Fig. 4).

Diagnostic value of CEUS patterns. Together, 95.5% of the lesions exhibiting a hyper−hypo pattern were malignant. The hyper−hypo pattern was significantly more common in HCC than in iCCA ($p = 0.02$), but also occurred in about one-third of the benign lesions. Early washout (≤60 s) in lesions with APHE was associated with malignancy, as this pattern was found in 65 malignant lesions (50 HCCs, 8 iCCAs, 7 other malignancies), but only one benign lesion (necrosis in vasculitis). As for the onset of washout, iCCAs exhibited early washout significantly more often in the portal venous phase ($p < 0.01$), whereas late-phase washout was associated with HCC ($p = 0.02$).

The positive predictive value were almost equal for the hyper−hypo and the hyper−iso patterns for the non-invasive diagnosis of HCC (86.8% vs. 85.4%).

DISCUSSION

Our results reaffirm the fact that APHE is the key diagnostic feature of HCC in CEUS; in our collective, it was present in 271 of 316 HCCs (85.8%). In fact, only 17 of 312 lesions with hyperenhancement in the arterial phase were benign (5.4%). Thus, the positive predictive value for malignancy of APHE was 94.6% in patients at risk for HCC (cirrhosis, 76.5%). On the other hand, these findings imply that about 14% of HCCs do not exhibit APHE on CEUS. Similar results have been reported by Forner et al. (2015) when assessing CEUS in 119 HCCs <2 cm in cirrhotic patients. The authors found a lack of arterial hyperenhancement in 15.1% of these lesions (Forner et al. 2015). However, in the collective of Forner et al. (2015), not all of the lesions were histologically proven, but some used magnetic resonance imaging (MRI) as the reference standard. In our analysis of histology-proven focal liver lesions, all three HCCs <10 mm in size and 29 of 34 HCCs 10−20 mm in size exhibited arterial hyperenhancement (86.5%). This is in accordance with earlier studies indicating no relationship between lesion size and the presence of APHE (von Herbay et al. 2009). Conversely, Giorgio et al. (2016), in assessing 229 focal liver lesions 7−20 mm in size with histology and MRI as the reference standard (199 HCCs), found APHE in 190 of 199 HCCs (95.5%), with a sensitivity of 94.5%, specificity of 100%, positive predictive value of 100% and negative predictive value of 76.9% (Giorgio et al. 2016). Moreover, the authors found that APHE was less frequently observed in small HCCs ≤10 mm (77% of HCCs vs. 98% of HCCs 11−20 mm in size). Thus, this discrepancy might be owing to the fact that Giorgio et al. included only small HCCs ≤2 cm. Similarly, Giorgio et al. (2016) obtained a specificity of 100% for arterial phase hyperenhancement, whereas our results suggest that APHE is not specific for HCC. In contrast, we also found APHE in >50% of the iCCAs and non-HCC and non-iCCA-malignancies and even in 50% of the benign lesions. In particular, 7 of 20 regenerative/dysplastic nodules (35%) exhibited APHE; 4 of these (20%) even had a hyper−hypo pattern.

With the characteristic CEUS pattern for HCC defined as APHE followed by mild washout in the late phase, <50% of the HCCs in our study collective had this pattern (148 of 316 HCCs). Of note, in 35 HCCs (11.1%), the onset of washout did not occur until after >4−6 min, emphasizing the importance of expanding the late phase if no contrast washout is observed. However, 13% of HCCs exhibited a hyper−iso pattern of APHE with no washout at all. These findings are in accordance with the study by Giorgio et al. (2016) mentioned earlier. In this study, 105 of 199 HCCs had a hyper−iso pattern (52.8%); the other 85 HCCs (42.7%) had a hyper−hypo pattern with onset of washout >3 min. In our study, the positive predictive value for HCC was 86% for the hyper−iso pattern and thus similar to that of the hyper−hypo pattern (87.4%). These results confirm the findings from a retrospective analysis by Leoni et al. (2013) assessing 155 focal liver lesions 10−30 mm in cirrhotic liver (among these were 71 primary and 56 recurrent HCCs). Leoni et al. observed a hyper−hypo pattern in 40.9% of HCCs with a diagnostic accuracy of 51% and a positive predictive value of 98%. A hyper−iso pattern was seen in 36.2% of HCCs, with a diagnostic accuracy of 77% and a positive predictive value of 94%. The positive predictive values of hyper−hypo and hyper−iso patterns did not differ.

Moreover, Leoni et al. (2013) observed an iso−iso pattern in 22.8% of the HCCs and 78.6% of the non-HCC lesions, indicating that the percentage of "atypical" HCCs on CEUS is even higher. These findings slightly differ from our results with arterial phase iso-enhancement seen in only 10% of the HCCs and about 23% of the non-HCC lesions. However, these results suggest that a relevant proportion of HCCs might escape the non-invasive diagnosis with CEUS defined by the hyper−hypo pattern. In this context, it must be taken into account that, compared with multiphase computed tomography (CT) and MRI, CEUS has a lower sensitivity for the detection of washout, especially in smaller HCCs between 10 and 20 mm in size (Furlan et al. 2012). Thus, some of the HCCs with a hyper−iso pattern on CEUS might exhibit a "typical" hyper−hypo pattern on CT or MRI. Concerning the onset of washout, recent studies suggest washout occurs earlier in non-HCC malignancies compared with HCC. Interestingly, 21.9% of the HCCs with a hyper−hypo pattern in our study had an onset of washout in the early portal venous phase after 1 min. In total, 50 of 65 lesions exhibiting early portal venous washout (76.9%) were HCCs, while 8 were iCCAs (12.3%). This implies, however, that early washout was a feature observed in 30.8% of iCCAs only. Similarly, in a retrospective single-center study assessing CEUS in 112 histologically proven HCCs 0.8−12.7 cm in size, Jang et al. (2007) reported APHE with subsequent contrast washout by 90 s in 42 lesions (43%), whereas late washout (defined as 91−180 s) occurred in 25 HCCs (26%) and very late washout (181−300 s) in 21 HCCs (22%). In this study, however, the typical enhancement pattern was defined as APHE followed by washout by 90 s, so that the late and very late onset of washout today considered specific to HCC was regarded as atypical by Jang et al. (2007). The intensity of washout was not the subject of this study. The intensity of washout is supposed to be mild in HCC, but marked in iCCA or metastases. However, in our study collective, the intensity of washout was graded as "marked" in

23.9% of HCCs (55 of 230) with APHE and washout. Contrarily, in the iCCA subgroup, mild and marked washout occurred with equal frequencies. Conversely, in a recent large retrospective study in five Italian centers assessing CEUS in 1006 focal liver lesions in 848 patients at risk for HCC, Terzi et al. (2018) reported early or marked washout in only 37 of 820 HCCs (4.5%). With the typical CEUS pattern of HCC defined as APHE followed by late (≥60 s) and mild washout, 515 HCCs (62.8%) exhibited a typical pattern. These discrepancies might at least partly be owing to the fact that in the study by Terzi et al., histological findings were available in 50.3% of the lesions only, whereas our study collective included histologically proven lesions only. Moreover, our study collective contained a relatively large proportion of lesions >2 cm, which is owing to the fact that patients were recruited prospectively in a real-life setting. The relatively large tumor size might have influenced the contrast enhancement pattern of the HCCs in our study.

In this context, it is important to bear in mind that the assessment of both the presence and intensity of washout in CEUS is inherently subjective, whereas in MRI or CT imaging, absolute intensities at defined points in time can be measured. Subjective judgment of the intensity of washout is an insufficient diagnostic tool in real life. As reported in recent interobserver studies (Schellhaas et al. 2018a, 2018b), the interobserver agreement for washout in CEUS is quite poor and inferior to that in MRI. On the other hand, the interobserver agreement for APHE in CEUS has been found to be substantial.

Concerning the subgroup of iCCA and non-HCC, non-iCCA malignancies, enhancement in the arterial phase varied, with iso-enhancement and hypo-enhancement occurring at similar frequencies. Strikingly, in all but one case with arterial hyperenhancement, contrast washout was present, suggesting that a hyper−iso pattern in cirrhosis is much more suggestive of HCC than of non-HCC malignancy. However, for differentiation between iCCA and other non-HCC malignancies, biopsy is mandatory.

A strength of the DEGUM HCC trial is the prospective multicenter study design in a real-life setting and the availability of histological findings in all lesions in this subanalysis. Of course, the inclusion of histologically proven lesions only introduces a selection bias, as today, many HCCs are diagnosed non-invasively according to HCC guidelines. However, for definite diagnosis and assessment of the diagnostic accuracy of imaging modalities, histology remains the gold standard and will be of increasing importance with the arrival of new potent drugs. Limitations of our study are the lack of data from long-term follow-up of the benign lesions, as this was not part of the study design. Thus, sampling errors or progression from dysplastic nodules to early HCCs was possible. In addition, about one-third of our study population consisted of larger lesions >5 cm, which may not represent the primary target population for non-invasive diagnosis by CEUS. However, one-third of the lesions were <3 cm, and the patient collective reflects a population from a clinical real-life setting.

CONCLUSIONS

Our results confirm that the specificity of the hyper−hypo pattern for the non-invasive diagnosis of HCC can be improved with the additional criteria of onset (late or very late) and intensity (mild rather than marked) of washout. Nonetheless, as this pattern was observed in only half of the HCCs in a real-life setting, further diagnostic workup remains inevitable in ambiguous cases. Especially in the context of evolving targeting drugs for HCC therapy, biopsy can be expected to gain further significance. Standardization of CEUS for focal liver lesions in patients at risk for HCC should include a very late phase contrast assessment after >4−6 min in cases with no contrast washout seen before, with an additional benefit of >10% of HCCs being diagnosed with contrast-enhanced ultrasound.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ultrasmedbio.2021.07.010.

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