HCCs lacking arterial phase hyperenhancement (APHE) on contrast-enhanced ultrasound (CEUS) – a diagnostic challenge. Findings from the prospective multicenter DEGUM CEUS HCC trial

Hepatozelluläre Karzinome (HCCs) ohne arterielles Hyperenhancement (APHE) in der Kontrastmittelsonografie (CEUS) – Erkenntnisse aus der prospektiven multizentrischen DEGUM-CEUS-HCC-Studie

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ABSTRACT

Objectives Hepatocellular carcinoma (HCC) upon contrast-enhanced ultrasound (CEUS) typically shows arterial phase hyperenhancement (APHE), followed by late (> 60 seconds) and mild contrast washout (WO). Although APHE is considered as the hallmark of HCC, it can be absent in some HCCs. Thus, we explored which sonomorphological and histopathological features of HCC are associated with a lack of APHE upon CEUS.

Methods Focal liver lesions in high-risk patients for HCC were assessed with CEUS following a standardized protocol in a prospective multi-center real-life setting. CEUS patterns in HCC were assessed, and tumour and patient characteristics were compared for HCCs with and without APHE.

Results 316 patients with HCC were recruited (cirrhosis, 76.9 %). APHE occurred in 271/316 HCCs (85.8 %). A lack of APHE was associated with portal vein thrombosis, tumour infiltration of the liver vessels ($p < 0.001$), larger size, multilocularity, and higher depth location upon ultrasound ($p < 0.01$). Histological grading did not differ between HCCs with and without APHE ($p = 0.39$). Histopathological features of HCCs without APHE included cirrhotic stromal reaction, marked tumour cell steatosis and absence of the typical surrounding dilated sinusoidal vascular channels.

Conclusion Correlation with histopathological findings support the fact that HCCs with a lack of APHE in CEUS are a heterogeneous group. The examiner has to be aware that HCCs with portal vein thrombosis or macro-invasion of the liver vessels may lack APHE.

ZUSAMMENFASSUNG

Ziele Hepatozelluläre Karzinome (HCCs) zeigen in der Kontrastmittelsonografie (CEUS) typischerweise ein arterielles Hyperenhancement (APHE) mit nachfolgend spätem (> 60 Sekunden), mildem Auswaschen (WO). Obwohl APHE das Hauptkriterium in der HCC-Diagnostik darstellt, fehlt dieses Merkmal bei einigen HCCs. Die vorliegende Arbeit sollte untersuchen, welche sonomorphologischen und histopathologischen Eigenschaften mit fehlendem APHE assoziiert sind.

Methoden Fokale Leberläsionen bei HCC-Hochrisikopatienten wurden nach einem standardisierten CEUS-Protokoll in einem prospektiven multizentrischen Ansatz untersucht. Die CEUS-Muster der HCCs wurden analysiert und Tumor- und Pa-

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tientencharakteristika für HCCs mit und ohne APHE verglichen.

Ergebnisse 316 HCC-Patienten wurden eingeschlossen (76,9 % Zirrhose). APHE fand sich bei 271/316 HCCs (85,8 %). Fehlendes APHE war assoziiert mit Pfortaderthrombose, Tumorinfiltration in die Lebergefäße ($p < 0{,}001$), größerem Tumordurchmesser, Multilokularität und größerer Tiefenlokalisation ($p < 0{,}01$). Der histologische Differenzierungsgrad unterschied sich nicht zwischen HCCs mit und ohne APHE ($p = 0{,}39$). Histologisch zeigten HCCs ohne APHE zirrhotische Stromareaktionen, ausgeprägte Tumorzellverfettung und ein Fehlen der typischen umgebenden erweiterten Sinusoide.

Schlussfolgerungen Die Korrelation mit histopathologischen Merkmalen belegt, dass HCCs mit fehlendem APHE im CEUS eine heterogene Gruppe darstellen. Der Untersucher sollte sich bewusst machen, dass APHE bei HCCs mit Pfortaderthrombose oder Makrogefäßinvasion fehlen kann.

Background

Hepatocellular carcinoma (HCC) in high-risk patients can be diagnosed non-invasively using contrast-enhanced ultrasound (CEUS), magnetic resonance imaging (MRI), or computed tomography (CT), if the typical enhancement pattern of arterial phase hyperenhancement (APHE) followed by contrast washout (WO) in the portal venous or late phase is present. Standardised CEUS algorithms such as CEUS LI-RADS (Contrast Enhanced UltraSound Liver Imaging Reporting And Data System) and ESCULAP (Erlanger Synopsis for Contrast-enhanced Ultrasound for Liver lesion Assessment in Patients at risk) allow a subtle classification of nodules using categories ranging from definitely benign nodules to definite HCC [1, 2, 3, 4]. According to CEUS LI-RADS, non-invasive diagnosis of HCC in CEUS relies on a combination of APHE followed by contrast washout of mild degree with an onset no earlier than ≥ 60 seconds after contrast injection. This definition is referred to in the European HCC guidelines by the European Association for the Study of Liver Diseases (EASL) [5]. However, there is evidence in the literature that some HCCs do not display the characteristic enhancement pattern of APHE followed by mild and late washout (hyper-hypo pattern) [6, 7, 8, 9, 10, 11, 12, 13, 14, 15].

Most recently, we conducted a prospective multicentre study funded by the German Society for Ultrasound in Medicine (DEGUM) to assess the diagnostic accuracy of standardised CEUS for the non-invasive diagnosis of HCC in high-risk patients in a real-life setting [3, 4]. Our results suggested that although arterial phase APHE can be considered the main characteristic feature of HCC in CEUS, some HCCs showed an atypical enhancement pattern with a lack of APHE. Therefore, the purpose of this *post hoc* sub-analysis was to identify characteristics of these HCCs without APHE in order to understand, why some HCCs might escape non-invasive diagnosis with CEUS in clinical routine.

Materials and Methods

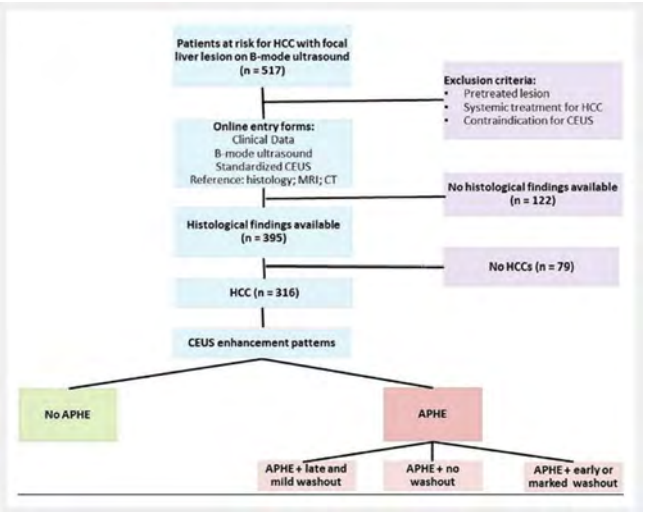
Study Design

The design of the DEGUM CEUS HCC study has been described in detail recently [3, 4]. Briefly, the study was conducted as a prospective multicentre study. Inclusion criteria were the presence of a risk factor for HCC according to national HCC guidelines, a solid focal liver lesion visible upon B-mode ultrasound, the availability of a reference standard (histology, or – if this was not possible – MRI or CT), and the patient's informed consent. Clinical and imaging data were entered via password-protected, individualised online accounts. The local Ethics committee approved the study (ethics vote 16_17B).

This manuscript deals with the features of typical versus atypical HCCs. The study collective was limited to those patients with histologically proven HCC. The design of this sub-analysis is shown in ▶ **Fig. 1**.

Standardized Contrast enhanced ultrasound

Contrast-enhanced ultrasound (CEUS) was performed following a standardized protocol [3, 4, 6]. Examiners were instructed to apply a second contrast bolus with subsequent assessment of the late phase only if contrast enhancement was insufficient in the late phase. The contrast enhancement of the index lesion relative to the surrounding parenchyma was recorded at the following time points: arterial phase (arrival of the first microbubbles until the maximum enhancement was reached in the lesion); early portal venous phase, at 60 seconds; late phase, at 3 minutes; very late phase, after 4–6 minutes. The last examination point could be omitted if contrast washout was visible at 3 minutes. If contrast washout was present, examiners had to classify its onset (early,



▶ **Fig. 1** illustrates the study design. CEUS, contrast-enhanced ultrasound; HCC, hepatocellular carcinoma; APHE, arterial phase hyperenhancement; MRI, magnetic resonance imaging; CT, computed tomography.

≤ 60 seconds; late, 3 minutes; very late, 4–6 minutes) and extent (mild versus marked).

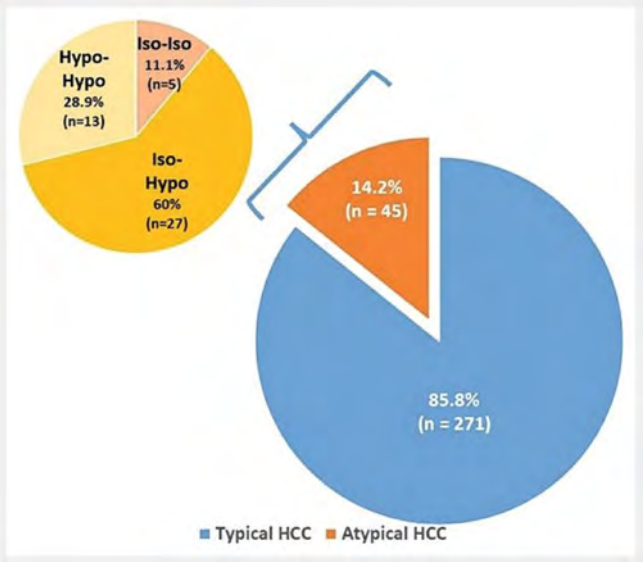
HCCs with and without APHE were compared in terms of patient characteristics (liver parenchyma, comorbidities) and tumour characteristics (histological grading, size, location).

In case of more than one lesion per patient, examiners were instructed to choose only the best accessible lesion for CEUS and further assessment.

Statistical Analysis

Data was exported from the online entry forms using Microsoft Excel. Quantitative variables were expressed as a mean and range. Categorical variables were expressed as frequencies. Groups were compared using the t-Test for continuous data, Pearson's Chi-squared test with Yates' continuity correction or Fisher's Exact Test for Count Data. Differences were considered significant for $p < 0.05$.

For tumour characteristics with $p < 0.1$, we performed a multivariable logistic regression. Lesion size and depth location were included as continuous variables.



▶ **Fig. 2** illustrates the proportion of HCC subgroups according to CEUS patterns. APHE, arterial phase hyperenhancement; iso, isoenhancement; hypo, hypoenhancement.

Results

Patient and tumour characteristics

316 patients with histologically proven HCC were recruited (male, n = 272; female, n = 44). Mean age was 67 years (range, 28–88 years). 243/316 patients had liver cirrhosis based on patients' history, clinical data or findings from imaging or histology (76.9 %), mostly in compensated stages (CHILD A, 72.4 %; CHILD B, 24.7 %; CHILD C, 2.9 %). Most patients were in a good general condition according to the East Cooperation Oncology Group (ECOG) performance score (ECOG 0, 63.3 %; ECOG 1–2, 35.1 %; ECOG 3–4, 1.6 %) [13]. 45 patients (14.2 %) had a history of extrahepatic malignancy. 124 (39.2 %) suffered from diabetes mellitus.

Concerning histological grading, most HCCs showed moderate differentiation (G2, 55 %; G1, 22 %; G3, 16 %); in 21 HCCs (7 %), information on grading was not available. Larger HCCs > 5 cm and those with diffuse infiltration of a liver lobe were more often poorly differentiated (G3, 21.5 % and 25 %, respectively), while smaller tumours ≤ 2 cm showed a tendency towards better differentiation (G1, 27 %; G3, 8.1 %); however, the level of statistical significance was not reached (**Supplemental Table 1**). Similarly, portal vein thrombosis rarely occurred in G1 HCCs (5.4 %), but mostly in moderately differentiated tumours (62.2 %; $p < 0.05$). Macro-invasion of the liver vessels was associated with poor differentiation (G3, 20.5 %), although again – due to the small subgroup size of G3 tumours – the level of statistical significance was not reached (**Supplemental Table 1**).

Subgroups of HCC according to CEUS patterns

▶ **Fig. 2** illustrates the subgroups according to CEUS patterns of the 316 HCCs.

APHE was seen in 271/316 HCCs (85.8 %). 45 HCCs showed a lack of APHE (14.2 %) and were therefore classified as atypical HCC. Atypical HCCs showed either isoenhancement or hypoenhancement in the arterial phase. The most common type in this subgroup was an iso-hypo pattern (n = 27) with mostly mild washout (96.3 %) and only one case of marked washout. As to the onset of washout, 5/27 HCCs with an iso-hypo pattern showed early washout ≤ 60 seconds (18.5 %); 19 showed late washout after 3 minutes (70.4 %), and three very late washout after > 4–6 minutes (11.1 %).

Characteristics of HCCs with and without APHE

Patient and tumour characteristics for HCCs with and without APHE are summarized in ▶ **Table 1** and ▶ **Table 2**. ▶ **Table 1** shows patient characteristics of HCCs with and without APHE in direct comparison. HCC, hepatocellular carcinoma; APHE, arterial phase hyperenhancement. ▶ **Table 2** shows tumour characteristics of HCCs with and without APHE in direct comparison. HCC, hepatocellular carcinoma; APHE, arterial phase hyperenhancement.

There was a male predominance in both subgroups (mean, 86 % male patients). There were no differences in the frequencies of liver cirrhosis, history of extrahepatic malignancies and diabetes mellitus between HCCs with and without APHE. In the subgroup of HCCs lacking APHE, there was a higher proportion of patients with a compromised general condition (ECOG 2–4); also, there were more patients with a transjugular intrahepatic portosystemic stent shunt (TIPSS) (both $p < 0.05$).

HCCs with APHE were more often solitary lesions compared to HCCs without APHE ($p < 0.01$), whereas HCCs lacking APHE tended towards higher depth location ($p < 0.01$). HCCs without APHE tended to be larger in size ($p < 0.01$). Macro-invasion of the liver vessels and portal vein thrombosis occurred significantly more often in HCCs without APHE ($p < 0.01$). There were no differences in grading between HCCs with and without APHE.

▶ **Table 1** Patient characteristics of HCCs with and without APHEus.

	HCCs with APHE (n = 271)	HCCs without APHE (n = 45)	Total (n = 316)	p-value*
Age (mean; range)	67.7 (28–88)	68.7 (45–88)	**67.9 (24–88)**	0.54
Male	233 (86 %)	39 (87 %)	**272 (86 %)**	1
Female	38 (14 %)	6 (13 %)	**44 (14 %)**	
ECOG				< 0.01
ECOG 0	181 (67 %)	19 (42 %)	**200 (63 %)**	
ECOG 1–4	90 (33 %)	26 (58 %)	**116 (37 %)**	
Liver cirrhosis	205 (76 %)	38 (84 %)	**243 (77 %)**	0.2
CHILD stage				0.17
A	152 (74.1 %)	24 (63.2 %)	**176 (72.4 %)**	
B–C	53 (25.9 %)	14 (36.8 %)	**67 (27.6 %)**	
Diabetes mellitus	106 (39 %)	18 (40 %)	**124 (39 %)**	1
Extrahepatic malignancy	41 (15 %)	4 (9 %)	**45 (14 %)**	0.36

APHE, arterial phase hyperenhancement, with or without subsequent washout (WO); HCC, hepatocellular carcinoma; ECOG, Eastern Cooperative Oncology Group performance status; TIPSS, transjugular intrahepatic portosystemic stent shunt.
*t-Test for continuous data, Fisher's Exact Test for Count Data.

Multivariate analysis showed statistical significance only for the parameters lesion size, diffuse tumour growth, depth location and portal vein thrombosis **(■ Supplemental Table 1)**. The classification of HCCs without APHE according to the standardised CEUS algorithm CEUS LI-RADS (Contrast-Enhanced UltraSound Liver Imaging Reporting and Data System) is shown in **■ Supplemental Table 2**.

Histopathological Characteristics of HCCs without APHE

Review of the histopathological slides for the HCCs without APHE from our centre (n = 26) revealed heterogeneous findings, and no single feature was found to correlate with the atypical CEUS pattern. However, remarkable findings included prominent desmoplastic or cirrhotic stromal reaction (observed in 6 cases), prominent tumour cell steatosis (observed in 6 cases) and absence of the typical dilated sinusoidal vascular channels surrounding the trabeculae and nests of tumour cells (seen in 4 cases).

▶ **Fig. 3** A–D shows histopathological findings in four examples of atypical HCCs with a lack of APHE in CEUS. Typical CEUS examples are shown in ▶ **Fig. 4**.

Discussion

Large retrospective studies have demonstrated that the typical enhancement pattern of HCC upon CEUS is APHE with late-onset (> 60 seconds), mild washout (definite HCC LR-5 according to the CEUS LI-RADS system) [7, 8, 9, 10, 11, 12, 13, 14, 15].

Although APHE has been shown to be the key diagnostic feature of HCCs upon CEUS, there is evidence from the literature that this criterion can be absent in up to 22 % of HCCs. The frequency of APHE observed in HCC varies from > 95 % in some studies to 77–85 % in others [2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. The observation of lacking APHE in HCC is also observed with MRI and CT. For LI-RADS MRI and CT, recent studies describe a lack of APHE in up to 25 % of histologically confirmed HCCs [18, 19].

Our prospective real-life data on CEUS now confirm that there is a relevant subgroup of HCCs (about 14 %) lacking this feature. As to the question of why such a significant proportion of HCCs do not display the characteristic APHE, our data show that there are several factors associated with atypical contrast enhancement patterns.

Perfusion alterations

Contrary to contrast media used in MRI and CT, the ultrasound contrast agent Sonovue used in Europe the United States and some parts of Asia is a purely intravascular agent. Thus, it seems reasonable that perfusion alterations of the liver parenchyma will affect contrast enhancement patterns using an intravascular contrast agent. First of all and probably most importantly, we noted portal vein thrombosis in 31.1 % and tumour infiltration of the liver vessels in 31.1 % of HCCs with a lack of APHE, versus 7 % and 9.5 %, respectively, in typical HCCs. This seems unexpected at first glance, as a reduced flow via the portal vein is known to increase arterial blood flow and should therefore result in a more pronounced arterial enhancement. However, it seems that the arterial enhancement of the surrounding tissue is rather increased in these cases, leading to assimilation of enhancement characteristics. Analogously, the presence of a transjugular intrahepatic portosystemic stent shunt (TIPSS) can affect the contrast enhancement pattern in HCC. A very recent monocentric study by Chang et al. reported APHE to occur earlier in HCC in the presence of a

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▶ **Table 2** Tumour characteristics HCCs with and without APHE.

	HCCs with APHE (n = 271)	HCCs without APHE (n = 45)	Total (n = 316)	p-value*
Lesion number				< 0.01
Solitary	175 (65 %)	21 (47 %)	**196 (62 %)**	
Multiple	81 (30 %)	11 (24 %)	**92 (29 %)**	
Diffuse	15 (6 %)	13 (29 %)	**28 (9 %)**	
Lesion size [mm] (median; range)	49 (6–200)	69 (12–210)	**52 (6–210)**	< 0.01
≤ 5 cm	182 (67 %)	19 (42 %)	**201 (64 %)**	< 0.01
> 5 cm	89 (33 %)	26 (58 %)	**115 (36 %)**	
Depth location [cm] (median; range)	6 (2–15)	8 (2–15)	**6 (2–15)**	< 0.01
Depth ≤ 5 cm	120 (44 %)	13 (29 %)	**133 (42 %)**	< 0.01
Depth > 5 cm	151 (56 %)	32 (71 %)	**183 (58 %)**	
Lesion echogenicity				0.07
Hyperechoic	61 (23 %)	6 (13 %)	**67 (21 %)**	
Isoechoic	60 (22 %)	17 (38 %)	**77 (24 %)**	
Hypoechoic	150 (55 %)	22 (49 %)	**172 (54 %)**	
Grading*				0.58
G1	63 (23 %)	8 (18 %)	**71 (22 %)**	
G2	150 (55 %)	23 (51 %)	**173 (55 %)**	
G3	42 (15 %)	9 (20 %)	**51 (16 %)**	
Macro-invasion of liver vessels (B-mode, colour-mode)	30 (11 %)	14 (31 %)	**44 (14 %)**	< 0.01
Bland portal vein thrombosis	22 (8 %)	14 (31 %)	**36 (11 %)**	< 0.01
Enhancing tumour thrombus	17 (6 %)	3 (7 %)	**20 (6 %)**	1

APHE, arterial phase hyperenhancement (APHE), with or without subsequent washout (WO). HCC, hepatocellular carcinoma; n.a., not available; Patients with no grading available (n = 16 / 5) were excluded from statistical analysis, therefore percentages do not add up to 100 %.
*t-Test for continuous data, Fisher's Exact Test for Count Data.

TIPSS, whereas contrast washout tended to occur more often and with a later onset in patients with a TIPSS [20]. However, of our sample size (two patients in the subgroup of typical HCC and six patients in the subgroup of atypical HCC) was too small for definite conclusions. Collectively, the different enhancement behaviour of HCCs seems not to be explicable by altered perfusion conditions alone, but other aspects such as tumour differentiation and biology might be implicated.

Influence of size and grading

Another factor associated with atypical enhancement behaviour of HCC might be differences in grading. While we initially speculated that a lack of APHE might be associated with large tumour size and moderate or poor differentiation, we did not find differences in grading between typical and atypical HCCs upon CEUS. This suggested that atypical HCCs might have a different tumour biology altogether, surpassing tangible differences in tumour size and histological grading.

There is considerable controversy in the literature on the impact of size and grading on contrast enhancement patterns in HCC. While some studies suggest evidence that typical enhancement patterns might be more common in moderately differentiated HCCs , others report no correlation between grading and APHE or WO [21, 22, 23].

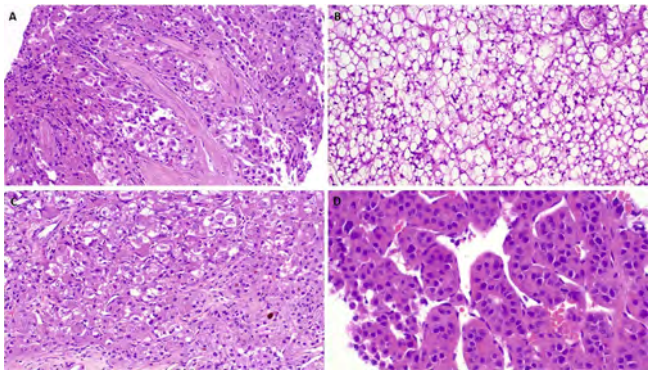
Nonetheless, all studies report a proportion of G2 HCCs far higher than that of G1 and G3 tumours; thus, these conclusions have to be interpreted cautiously. Similarly, studies diverge on a possible impact of size on enhancement behaviour, with some authors suggesting a correlation between APHE and / or WO and larger tumour size [21, 22, 23]. However, these associations are often significant for certain subgroups, and there are considerable discrepancies in the size of tumours assessed as well as sample sizes of the different subgroups.

Lack of APHE in correlation to histopathological findings

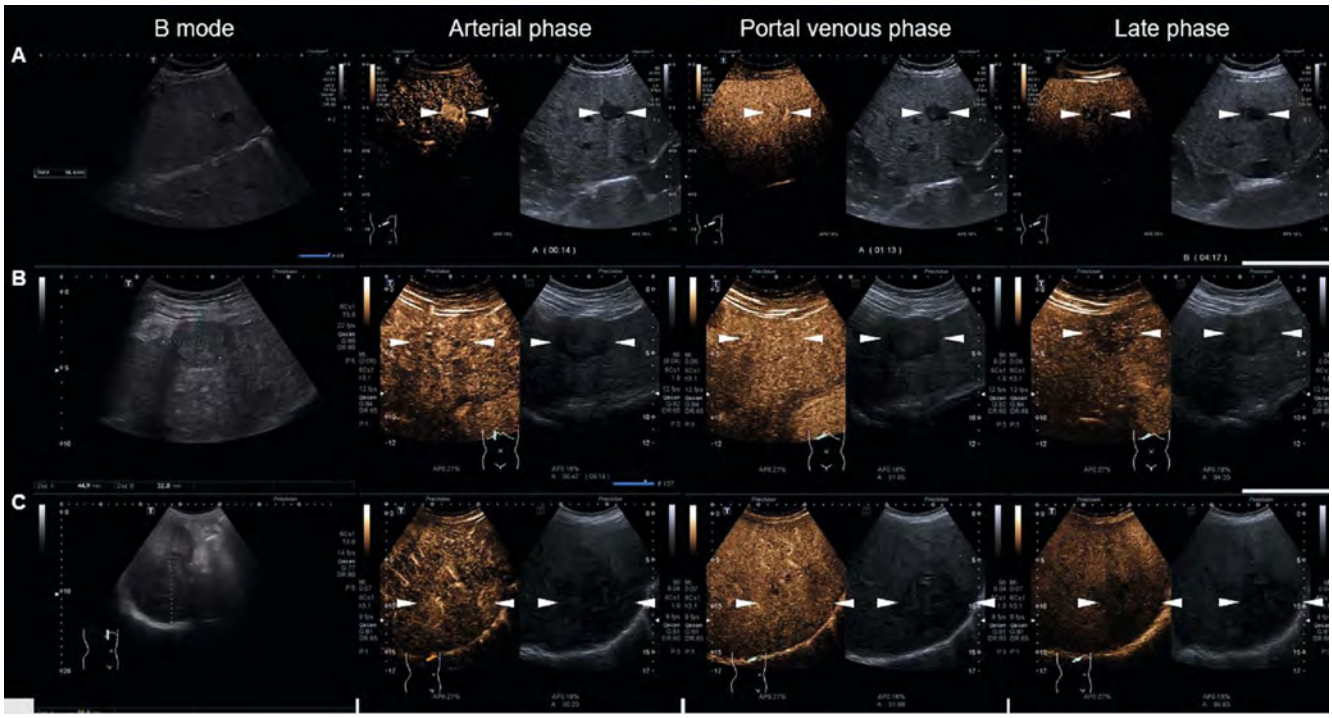
Although recognized as the key feature of HCC upon CEUS, 14.2 % of the HCCs in our study collective lacked APHE. This feature was significantly associated with portal vein thrombosis, macro-invasion of the liver vessels, larger tumour size, diffusely infiltrating

tumours and multilocular growth, suggesting a different and potentially more aggressive tumour biology in this subtype (▶ **Table 2**). A lack of APHE seemed to be associated with poorer tumour differentiation (17.8 % G1 / 20 % G3 in HCC with lack of APHE versus 23.4 % G1 / 17.1 % G3 in typical HCC), however lacking significance due to small sample size of the subgroups. Conversely, there is evidence from retrospective single-centre studies for a possible correlation between APHE and moderate or poor tumour differentiation [21, 22]. For instance, a recent retrospective single-centre study in China in 372 histologically proven HCCs ≤ 30 mm in 346 patients (cirrhosis, 59.5 %) reported a significant association of APHE with moderate or poor tumour differentiation (96.2 % in moderately / poorly differentiated HCCs versus 58.6 % in well-differentiated HCCs) [22]. The authors suggest that their finding of more frequent APHE in larger and progressed HCCs could be due to the fact that the smaller and well differentiated, early HCCs might not have fully developed neoangiogenesis with the so-called unpaired arteries yet. However, the grading system applied in this study followed the scheme by Edmondson and Steiner with a distinction between early / well-differentiated HCC (grade I), corresponding to G1 in our study, and progressed / moderately or poorly differentiated HCC (grade II-IV), corresponding to G4 in our study. Compared to our patient collective, the proportion of well-differentiated HCCs was smaller in the study by Fan et al. (grade I, 7.8 %; grade II, 47.6 %; grade III, 43.5 %; grade IV, 1.1 %) [22]. Moreover, the patient collective in this study consisted mainly of patients with chronic hepatitis B infection (90.2 %) with only 59.5 % of the patients suffering from cirrhosis [22]; thus, the results may not be completely transferrable to a Caucasian population with liver cirrhosis as the main risk factor of HCC. Altogether, although some studies suggest a correlation between moderate or poor tumour differentiation and a lack of APHE in HCC, this issue remains controversial.

Correlation of the CEUS findings with histopathological characteristics revealed that atypical contrast enhancement behaviour upon CEUS cannot be related to one single histological feature.



▶ **Fig. 3 A–D**: Representative histological examples of atypical HCCs. **A**, infiltrative solid tumour nests with desmoplastic stroma reaction. **B**, extensive steatotic tumour with poor vascularization of the stroma. **C**, mildly steatotic hypovascularized tumor. **D**, classical example of HCC showing the characteristic sinusoidal vascular spaces encasing the neoplastic trabeculae, note absence of visible connective tissue stroma in the background. Note that examples A, B and C all lacked the sinusoidal vasculature.



▶ **Fig. 4** Representative CEUS examples of HCCs with and without APHE. **A**, typical HCC: APHE, followed by isoenhancement in the portal venous phase and mild washout in the late phase. B and C, atypical HCCs lacking APHE. **B**, arterial phase isoenhancement, sustained isoenhancement during the portal venous phase, contrast washout in the late phase. **C**, arterial phase isoenhancement, sustained isoenhancement during the portal venous phase, mild washout in the late phase.

Furthermore, the group of HCCs with no APHE was found to be very heterogeneous. Features found in several atypical HCCs, which might in part explain the lack of APHE, included prominent desmoplastic or cirrhotic stromal reaction (6 out of 45 atypical HCC = 13.3 %), prominent tumour cell steatosis (6/45, 13.3 %) and absence of the typical dilated sinusoidal vascular channels surrounding the trabeculae and nests of tumour cells (4/45, 8.9 %). Thus, although there is no pathohistological feature common to all atypical HCCs, we could identify some characteristics that might cause a lack of APHE.

Finally, a potential technical limitation needs to be discussed. HCCs with a lack of APHE showed a slight tendency towards higher depth location, suggesting the possibility that this deeper location might have been a technical limitation. However, the imaging quality in this subgroup was judged as sufficiently, thus this possibility seems unlikely.

Furthermore, as HCCs in cirrhotic liver can be diagnosed non-invasively if the typical contrast enhancement pattern is present, there might be some sort of selection bias because also in the group of typical HCCs with APHE, only lesions undergoing biopsy or resection were enrolled. However, this was inevitable as our study design used histology as the gold standard. Also, we are aware that histopathological characteristics of HCCs without APHE could be provided in only 26 patients, making our conclusion of great interest, but possibly not definitive. A larger patient population would be desirable, but unfortunately, given the multicentre design of our study, it was not possible to review the histological samples of all other centres.

The search for any histological correlation with washout features, such as no washout, early or marked washout, late and mild washout, is also an important issue to assess beyond APHE, and we plan to analyze our data for types of washout in a separate manuscript.

Conclusion

Although defined as typical of HCC in CEUS, the enhancement pattern of APHE followed by late-onset (> 60 seconds), the examiner should be aware that especially in the case of large tumour size, portal vein thrombosis and tumour infiltration of the liver vessels, the hallmark of APHE can be missing in HCC. Correlation with histopathological findings indicate that the HCCs with a lack of APHE are a heterogeneous group. However, some features were found to occur in a relevant proportion of atypical HCCs, such as prominent desmoplastic or cirrhotic stromal reaction, marked tumour cell steatosis and absence of the typical surrounding dilated sinusoidal vascular channels. With the emerging drug developments for targeted therapies in HCC, biopsy can be expected to gain further significance both from a diagnostic and therapeutic point of view.

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Conflict of Interest

The authors declare that they have no conflict of interest. ■ correct? ■

Clinical Trial

Registration number (trial ID): NCT03405909 | Trial registry: ClinicalTrials.gov (http://www.clinicaltrials.gov/) | Type of Study: prospective multicentre

References

[1] van der Pol CB, McInnes MDF, Salameh JP et al. CT/MRI and CEUS LI-RADS Major Features Association with Hepatocellular Carcinoma: Individual Patient Data Meta-Analysis. Radiology 2022; 302 (2): 326–335

[2] Schellhaas B, Goertz RS, Pfeifer L et al. Diagnostic accuracy of contrast-enhanced ultrasound for the differential diagnosis of hepatocellular carcinoma: ESCULAP versus CEUS-LI-RADS. Eur J Gastroenterol Hepatol 2017; 29 (9): 1036–1044

[3] Strobel D, Jung EM, Ziesch M et al. Real-life assessment of standardized contrast-enhanced ultrasound (CEUS) and CEUS algorithms (CEUS LI-RADS/ESCULAP) in hepatic nodules in cirrhotic patients-a prospective multicenter study. Eur Radiol 2021; 31 (10): 7614–7625. doi:10.1007/s00330-021-07872-3

[4] Schellhaas B, Bernatik T, Dirks K et al. 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. Ultrasound Med Biol 2021; 47 (11): 3170–3180. doi:10.1016/j.ultrasmedbio.2021.07.010

[5] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2018; 69 (1): 182–236

[6] Schellhaas B, Strobel D. Tips and Tricks in Contrast-Enhanced Ultrasound (CEUS) for the Characterization and Detection of Liver Malignancies. Ultraschall in Med 2019; 40 (4): 404–424. doi:10.1055/a-0900-3962

[7] Giorgio A, Montesarchio L, Gatti P et al. Contrast-Enhanced Ultrasound: a Simple and Effective Tool in Defining a Rapid Diagnostic Work-up for Small Nodules Detected in Cirrhotic Patients during Surveillance. J Gastrointestin Liver Dis 2016; 25 (2): 205–211

[8] Boozari B, Soudah B, Rifai K et al. Grading of hypervascular hepatocellular carcinoma using late phase of contrast enhanced sonography – a prospective study. Dig Liver Dis 2011; 43: 484–490. doi:10.1016/j.dld.2011.01.001

[9] Leoni S, Piscaglia F, Granito A et al. Characterization of primary and recurrent nodules in liver cirrhosis using contrast-enhanced ultrasound: which vascular criteria should be adopted? Ultraschall in Med 2013; 34 (3): 280–287

[10] Terzi E, Iavarone M, Pompili M et al. Contrast ultrasound LI-RADS LR-5 identifies hepatocellular carcinoma in cirrhosis in a multicenter restrospective study of 1,006 nodules. J Hepatol 2018; 68 (3): 485–492. doi:10.1016/j.jhep.2017.11.007

[11] Oken MM, Creech RH, Tormey DC et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5 (6): 649–655

[12] Fraquelli M, Nadarevic T, Colli A et al. Contrast-enhanced ultrasound for the diagnosis of hepatocellular carcinoma in adults with chronic liver disease. Cochrane Database Syst Rev 2022; 9 (9): CD013483

[13] Shin SK, Kim YS, Choi SJ et al. Contrast-enhanced ultrasound for the differentiation of small atypical hepatocellular carcinomas from dysplastic nodules in cirrhosis. Dig Liver Dis 2015; 47 (9): 775–782

[14] Forner A, Vilana R, Bianchi ER et al. Lack of arterial hypervascularity at contrast-enhanced ultrasound should not define the priority for diagnostic work-up of nodules <2 cm. J Hepatol 2015; 62 (1): 150–155

[15] Strobel D, Seitz K, Blank W et al. Tumor-specific vascularization pattern of liver metastasis, hepatocellular carcinoma, hemangioma and focal nodular hyperplasia in the differential diagnosis of 1,349 liver lesions in contrast-enhanced ultrasound (CEUS). Ultraschall in Med 2009; 30 (4): 376–382. doi:10.1055/s-0028-1109672

[16] Schellhaas B, Pfeifer L, Kielisch C et al. Interobserver Agreement for Contrast-Enhanced Ultrasound (CEUS)-Based Standardized Algorithms for the Diagnosis of Hepatocellular Carcinoma in High-Risk Patients. Ultraschall in Med 2018; 39 (6): 667–674. doi:10.1055/a-0612-7887

[17] Schellhaas B, Bernatik T, Bohle W et al. DEGUM CEUS HCC Study Group. Contrast-Enhanced Ultrasound Algorithms (CEUS-LIRADS/ESCULAP) for the Noninvasive Diagnosis of Hepatocellular Carcinoma – A Prospective Multicenter DEGUM Study. Ultraschall in Med 2021; 42 (2): e20

[18] Ehman EC, Behr SC, Umetsu SE et al. Rate of observation and inter-observer agreement for LI-RADS major features at CT and MRI in 184 pathology proven hepatocellular carcinomas. Abdom Radiol (NY) 2016; 41 (5): 963–969. doi:10.1007/s00261-015-0623-5

[19] Ludwig DR, Fraum TJ, Cannella R et al. Hepatocellular carcinoma (HCC) versus non-HCC: accuracy and reliability of Liver Imaging Reporting and Data System v2018. Abdom Radiol (NY) 2019; 44 (6): 2116–2132. doi:10.1007/s00261-019-01948-x

[20] Chang J, Dumitrache A, Böhling N et al. Alteration of contrast enhanced ultrasound (CEUS) of hepatocellular carcinoma in patients with cirrhosis and transjugular intrahepatic portosystemic shunt (TIPS). Sci Rep 2020; 10 (1): 20682

[21] Jang HJ, Kim TK, Burns PN et al. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. Radiology 2007; 244 (3): 898–906

[22] Fan PL, Ding H, Mao F et al. Enhancement patterns of small hepatocellular carcinoma (≤ 30 mm) on contrast-enhanced ultrasound: Correlation with clinicopathologic characteristics. Eur J Radiol 2020; 132: 109341

[23] von Herbay A, Vogt C, Westendorff J et al. Correlation between SonoVue enhancement in CEUS, HCC differentiation and HCC diameter: analysis of 130 patients with hepatocellular carcinoma (HCC). Ultraschall in Med 2009; 30 (6): 544–550