

# Evaluation of posttreatment response of hepatocellular carcinoma: comparison of ultrasonography with second-generation ultrasound contrast agent and multidetector CT

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## Abstract

We evaluated the ability of one-month follow-up contrast-enhanced ultrasound (CEUS) with second-generation contrast agent in monitoring radio frequency ablation (RFA) and transcatheter arterial chemoembolization (TACE) treatments of hepatocellular carcinoma (HCC). One-hundred forty-eight HCCs were studied using CEUS: 110 nodules were treated with RFA [41/110 RFA were performed using a pretreatment and an immediate postablation evaluation using CEUS (group 1); 69/110 using only US guidance (group 2)] and 38 nodules treated with TACE. For statistical analysis, McNemar test was used. Overall complete response was observed in 107/148 nodules (92/110 treated with RFA and 15/38 with TACE). A better rate of complete response was found in group 1 compared to group 2 (92.7% vs. 78.3%). In RFA treatment, CEUS showed a sensitivity of 83.3% and a specificity of 100% (diagnostic accuracy of 97%) using MDCT as reference standard with no statistical difference ( $p > 0.05$ ). CEUS detected all cases of incomplete response in HCC treated with TACE using angiography as reference standard (diagnostic accuracy 100%). We recommend assessing residual intratumoral flow on CEUS during RFA procedure to determine the necessity of immediate additional treatment. In case of positive CEUS results, HCC treated with TACE should be considered still viable.

**Key words:** Contrast-enhanced ultrasound—Radiofrequency ablation—Hepatocellular carcinoma—Transcatheter arterial chemoembolization—Computed tomography

Hepatocellular carcinoma (HCC) has an incidence which is increasing worldwide, represents more than 5% of all cancers, and estimated annual number of cases exceeds 500,000 [1].

If diagnosed at an early stage, patients should be considered for surgical resection and liver transplantation, which are considered to be potentially curative options; however, less than 30% of cases are candidates to surgery at the time of diagnosis due to advanced tumor stage and underlying liver cirrhosis [2]. For this condition, many patients should be considered for nonsurgical treatment such as radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE), percutaneous ethanol injection, etc. [1].

Accuracy in assessing treatment response is essential for determining the necessity of additional therapy to complete the treatment.

Multiphasic multidetector CT (MDCT) is one of the most commonly used modalities for assessing the therapeutic response to nonsurgical treatment in patients with HCC [3–5].

Several authors have applied contrast-enhanced ultrasound (CEUS) to evaluate the therapeutic response in HCCs treated with nonsurgical procedures [6–9].

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However, many authors used a first-generation contrast agent that has several procedural limits: in fact, interval-delay scanning or manual flash imaging is necessary. These limits were overcome by the introduction of second-generation contrast agents that allow real-time imaging during different vascular phases [10, 11].

Moreover, it has been proposed to use CEUS before, during, and immediately at the end of RFA procedure to assess the therapeutic results prior to closing the treatment session [12].

Aim of this prospective study was to assess the reliability of ultrasonography with a second-generation ultrasound contrast agent in evaluating HCC treated with TACE and RFA guided and monitored with CEUS.

## Methods

### *Patients*

*Institutional Review Board.* The study protocol was approved by the Local Ethics Committee; written informed consent was obtained before study inclusion from all patients.

*Population.* From February 2005 to December 2007, 162 consecutive patients [age range 43–75 years, mean age  $59 \pm 8.4$  (standard deviation) years] were referred to our Radiology Department to perform 1-month CEUS and MDCT examination for follow-up of nonsurgical-treated HCC.

Of 162 patients, 120 were treated with RFA and 42 were treated with TACE.

*Inclusion and exclusion criteria.* An inclusion criterion was a single treatment with RFA or TACE.

Exclusion criteria were the lack of identification of the HCC in gray-scale sonography and contraindication to iodinated contrast agents (allergic reactions; impaired renal function).

Excluded patients were: 14 for a history of multiple treatments with TACE or RFA, 7 for the lack of identification of the HCC in gray-scale sonography, and 2 for contraindication to iodinated contrast agents (allergic reactions,  $n = 1$ ; impaired renal function,  $n = 1$ ).

*Study population.* Of the total study population, 110 patients underwent RFA and 29 patients underwent TACE treatment.

In 9 patients with multiple-treated HCC (all treated with TACE), the two largest lesions were selected for this study. The other 130 patients had a single-treated HCC. Thus, a total of 148 nodules (110 treated with RFA and 38 nodules treated with TACE) were pooled for the study.

*Treatment design.* The medical records of the study population related to RFA design were reviewed by a radiologist.

In 41 of the 110 patients RFA treatments were performed using a pretreatment and an immediate postablation evaluation of the targeted HCC using CEUS. If even questionable residual tumor foci with enhancement or vascular supply were depicted, immediate CEUS-guided targeted re-treatment was carried out. These 41/110 radio frequency-ablated HCCs constituted group 1.

In 69 of the 110 patients RFA treatments were performed using real-time US guidance and the immediate postablation evaluation using CEUS was not performed. These 69/110 radio frequency-ablated HCC constituted group 2.

A flow diagram of patients' selection and treatment design is shown in Fig. 1.

### *CEUS examination*

All recruited subjects were evaluated with CEUS targeted to the index lesion.

Patients were scanned using an HDI 5000 scanner (Advanced Technology Laboratories, Bothell, WA) with a convex array probe (5-2 MHz) and a ProSound SSD-5500 scanner (Aloka, Tokyo, Japan) with a convex array probe (5-2 MHz). Pulse inversion harmonic imaging (PIHI) with low mechanical index ( $MI < 0.09$ ) and pure harmonic detection with low mechanical index ( $MI < 0.08$ ) were used.

Prior to injection of the contrast agent, a scanning plane displaying both the tumor and some surrounding liver parenchyma with fundamental B-mode was chosen before switching to contrast-dedicated software.

CEUS was performed using sulfur hexafluoride microbubbles (SonoVue®, Bracco, Milan). A bolus of 2.4 mL of SonoVue® was injected by hand via a 20-gauge intravenous cannula placed in the right antecubital vein at a rate of approximately 1 mL/s, followed by a 5-mL normal saline flush.

After SonoVue® injection, tumor was scanned for enhancement for 180 s with the patients holding their breath for a few seconds if necessary (observation of arterial, portal, and equilibrium phases).

### *MDCT examination*

Four-phase helical CT, including both nonenhanced and contrast-enhanced three-phase imaging was performed with multidetector row CT with four detectors (MX 8000, Philips Medical System, Eindhoven, the Netherlands). The images were obtained in the cranio-caudal direction, from the lower chest to the level of the iliac crest. The scanning parameters were 120 kVp, 200 mAs, slice thickness 6.5 mm, and pitch of 1.

CT scans were obtained: first at 30–35 s (arterial phase), at 70–75 s (portal phase), and then at 180 s (late phase) after intravenous injection of contrast material.

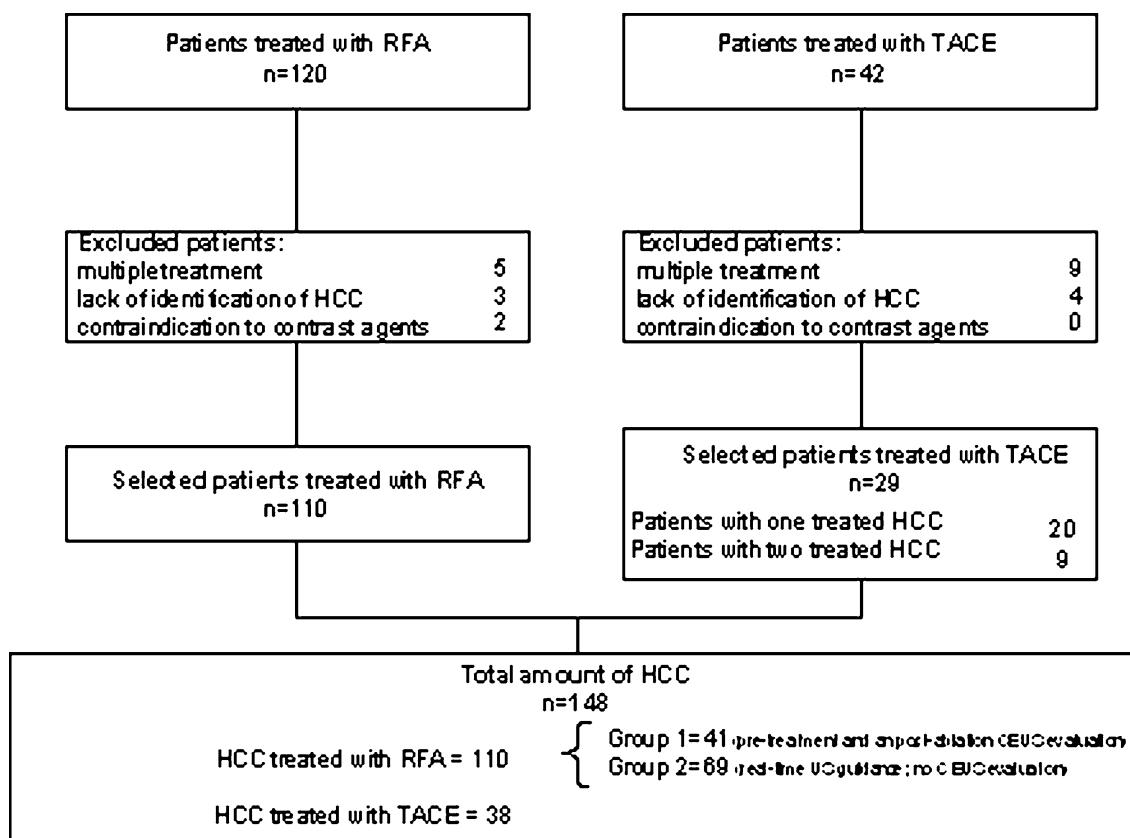


Fig. 1. Flow chart of study population.

A nonionic contrast agent material, 120–140 mL of iopromide (Ultravist 370, Schering, Berlin, Germany), was administered via a mechanical power injector (MK-IV Medrad, Pittsburgh, PA) via a 20-gauge intravenous cannula placed in an antecubital vein at a rate of 4–5 mL/s.

### Image analysis

To minimize the procedural variations, CEUS was performed by the same physician by using the same examination protocol. The imaging data were recorded on videotapes or on ultrasound device hard disk and reviewed by two independent experienced specialists, one being the radiologist who performed the examination. The two readers were blinded to each other's findings. Differences in detection of nodules enhancement were resolved by consensus. The interobserver agreement between two blinded readers was also evaluated.

Positive enhancement was defined as strong gray-scale enhancement appearing within the tumor. Positive enhancement was interpreted as viable tumor in treated nodules (incomplete response). In contrast, no enhancement of the lesion was defined as no-bubble signal within the tumor while the surrounding liver parenchyma was

filled with bubble signals. No enhancement was interpreted as complete tumor necrosis (complete response).

CEUS findings were compared with findings at MDCT by one radiologist.

At MDCT, a radiologist evaluated tumor enhancement by using the comparison of all triple-phase dynamic CT scans with nonenhanced CT scans. The radiologist did not have knowledge of the CEUS results. The interpretation of CT scans was performed by using film copies retrospectively.

Evaluations of treatment response on MDCT scans were as follows:

- RFA: a complete response was diagnosed when a hypoattenuating area portraying nonenhancement was present in both the arterial and portal venous phases in nodules after treatment. Conversely, an incomplete response was diagnosed when enhanced areas in the arterial phase within the tumor were present in nodules.
- TACE: a complete response was diagnosed when a complete homogeneous retention of iodized oil was present; incomplete response was diagnosed when incomplete retention of iodized oil with hypoattenuating areas showing enhancement was present in the arterial phase.

### Reference standard

One-month MDCT was used as reference standard in the evaluation of HCC that underwent RFA.

In HCC treated with TACE with incomplete response, an angiography with an additional TACE treatment was performed. In these cases, angiography was used as reference standard.

### Statistical analysis

Sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of CEUS after RFA and of CEUS and MDCT after TACE at 1 month were calculated.

Posttreatment tumor vascularity was compared with the gold standard (MDCT for RFA treatment and angiography for TACE treatment) by means of McNemar test; results are expressed as point estimates and 95% confidence intervals (95% CI).

Interobserver agreement was evaluated by means of  $k$ -coefficient. Statistical significance was set at 0.05, for a two-sided test.

The analysis was performed with the Statistical Analysis System (SAS) software package, Version 8.20 (SAS Institute, Cary, NC).

## Results

All patients tolerated the ultrasound contrast agent as administered without signs of adverse reactions or side effects.

### Treatment response

Overall, in 107 nodules, a complete response was observed (Fig. 2), whereas the other 41 nodules showed an incomplete response (Fig. 3).

The percentage of complete response was significantly higher in patients who underwent RFA than in those undergoing TACE (92/110–83.6% vs. 15/38–39.4%; McNemar test = 46.3; 1 df;  $p < 0.05$ ).

When considering only RFA, there were complete responses in both group 1 [38/41 (92.7%)] and group 2 [54/69 (78.3%)], with a statistically significant difference (McNemar test = 45.6; 1 df;  $p < 0.0001$ ).

### Accuracy of CEUS to define RFA efficacy

CEUS performed 1 month after treatment detected 15 of 18 incomplete response. This represents a sensitivity of 83.3%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 96.8%.

For HCC treated with RFA, results of CEUS agreed with those of the reference standard (MDCT) in 107/110 nodules, thus reaching a diagnostic accuracy of 0.97 (95% CI: 0.92–0.99) with no statistical difference (McNemar test  $\chi^2 = 1.33$ ; 1 df,  $p > 0.05$ ).

The agreement with the reference standard was 100% (41/41) in RFA group 1 and 95.6% (66/69) in RFA group 2.

Disagreement was due to the presence of three false-negative results on CEUS: two of three false-negative results were obtained in tumor deeply located, the other one in a subcapsular lesion located in segment 7 and not in a deep location (Fig. 4).

### Accuracy of CEUS to define TACE efficacy

MDCT performed 1 month after treatment detected 20 of 23 incomplete response. This represents a sensitivity of 86.9%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 83.3%.

CEUS performed 1 month after treatment detected all cases of incomplete response.

For HCC treated with TACE, results of CEUS and MDCT agreed with those of the reference standard (angiography) in 38/38 nodules (100%) [diagnostic accuracy 1 (95% CI: 0.91–0.99)] and in 35/38 (92.1%) nodules [diagnostic accuracy 0.92 (95% CI: 0.79–0.97)], respectively. No statistically significant differences between CEUS and angiography and between MDCT and angiography (McNemar test  $\chi^2 = 1.33$ ; 1 df,  $p > 0.05$ ) were found.

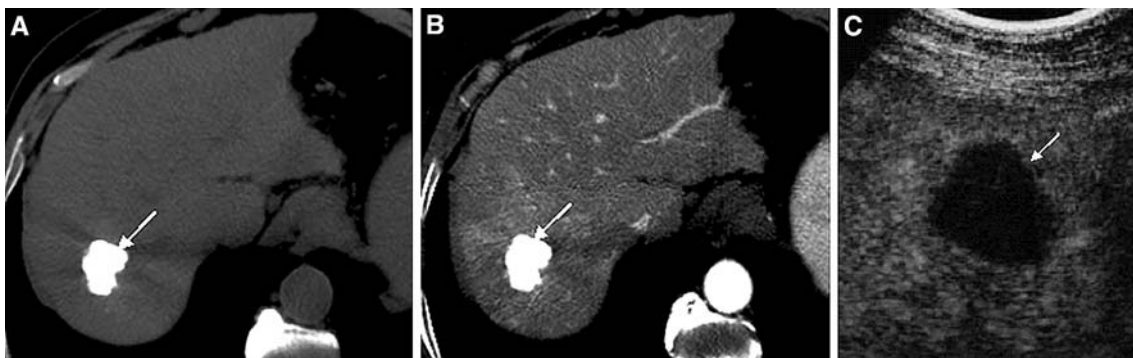


Fig. 2. Sixty-five-year-old woman with HCC treated with TACE therapy. (A) Nonenhanced CT scan and (B) arterial phase CT scan show a complete homogeneous retention of iodized oil (arrow). (C) CEUS shows no enhancement within the lesion (arrow).



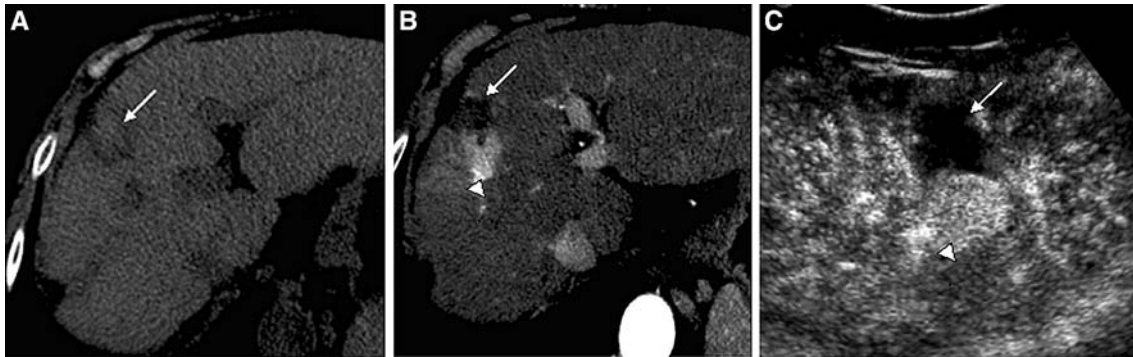


Fig. 3. Fifty-eight-year-old man with HCC treated with RFA therapy. (A) Nonenhanced CT scan, (B) arterial phase CT scan, and (C) CEUS (arterial phase) show positive

enhancement (*arrowhead*) in the lower portion of the nodule (viable tumor) and no enhancement (*arrow*) in the upper part of lesion, respectively.



Fig. 4. Fifty-eight-year-old man with HCC treated with RFA therapy. (A) Arterial phase CT scan shows hypervascular rim (*arrowhead*) of viable tumor in a nonenhancing hypodense portion of tumor (*arrow*) adequately ablated. (B) Late-phase

CT scan shows washout of the hypervascular rim (*arrowhead*) confirming the presence of viable tumor. (C) CEUS (arterial phase) shows no enhancement within the lesion (*arrow*).

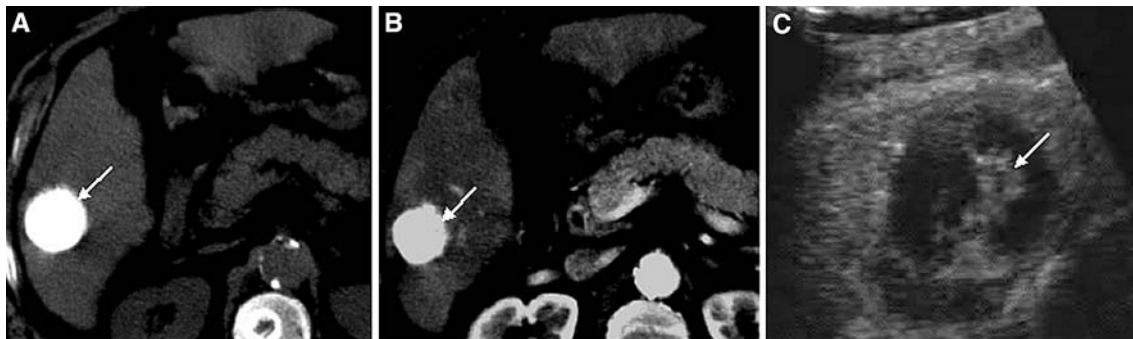


Fig. 5. Sixty-three-year-old man with HCC treated with TACE therapy. (A) Nonenhanced CT scan and (B) arterial phase CT scan show complete retention of iodized oil within the nodule (*arrow*), which suggests a complete response to

TACE therapy. (C) After CEUS, a strong gray-scale enhancement (*arrow*) appears within the tumor in arterial phase.

Disagreement was due to the presence of three false-negative results on MDCT. These three lesions showed a complete homogeneous retention of iodized oil (Fig. 5).

The 23 nodules treated with TACE which showed incomplete response (20 showed on MDCT and CEUS and 3 on CEUS exclusively) underwent an additional

TACE treatment (45–60 days), which resulted in a complete necrosis.

#### *Interobserver agreement*

Disagreement was observed in the analysis of only four nodules; however, by reviewing videotapes or video clips,

a consensus was obtained. The kappa coefficient for interobserver variability for CEUS evaluation was 0.93 (95% CI: 0.86–1.00), suggesting an excellent agreement.

## Discussion

In our series, HCCs treated with RFA showed a complete response in 83.6% of cases. This was a lower rate than reported in several previous studies, in which it was varying between 86% and 96% [13–16]. An explanation for this difference could be that all these studies (including ours) are inhomogeneous with regard to technology, treatment protocol, and follow-up period. For example, immediate postablation evaluation using CEUS and subsequent immediate CEUS-guided targeted re-treatment drastically increases the rate of complete response: in our series, by using this technique, a complete response was obtained in 92.7% (group 2) vs. 78.3% (group 1). This is not surprising; in fact, several studies reported that the introduction of intraoperative CEUS led to a reduction of the rate of partially unablated tumors (i.e., from 16.1% to 5.9% in the study of Solbiati et al. [12]).

The reported recurrent free rates of HCC treated by TACE were 28.3–67.5%. These results are strictly dependent on the number of repetition of TACE treatment, on tumor size, and on follow-up examination time [17–19]. In the study of Miraglia et al. [19], a single TACE procedure was sufficient to induce a complete tumor necrosis only in 69 of 162 (43%) patients. According to this result, our rate of complete necrosis after a single TACE treatment was 39.4%.

After treatment with RFA, 15 of 18 cases of treatment failure were correctly identified using CEUS. We found a close correlation between findings of CEUS and those of MDCT in revealing the outcome of thermal ablation treatment of HCC lesions. In all patients with a complete tumor response that was visible on MDCT images, viable tumors were no longer detectable on CEUS.

In 3 of the 18 cases of treatment failure, areas of viable tumor, which closely matched portions of tumor showing persistent enhancement on MDCT scans, were not revealed by CEUS.

Several studies [7, 20] showed that tumor location limited the visualization of lesions by CEUS. We believe that lesion location may have affected the results in the three false-negative cases; in fact, of these three lesions, one was subcapsular lesion and the other two were deeply seated lesions with a depth more than 6 cm from the transducer.

Regarding accuracy of CEUS to define TACE efficacy, our results show that MDCT was less sensitive for detecting residual vascular enhancement in HCC nodules after TACE than either sonographic method. CEUS accurately revealed the enhancement from the residual

viable portion (incomplete response) in all cases of the examined HCC nodules after TACE. MDCT and CEUS, compared with angiography, showed a sensitivity of 86.9% and 100% in identifying the presence of residual tumor, respectively.

Three of the 38 nodules that showed no enhancement on MDCT appeared partially enhanced on CEUS, whereas no nodule without enhancement on CEUS appeared partially enhanced on MDCT; three false-negative cases at MDCT were found compared with CEUS that detected all cases of incomplete response. Digital angiography examination of the three false-negative MDCT nodules confirmed the incomplete response.

The detection of the intratumoral blood flow after the chemoembolization using CEUS were much superior to the rates of detection using MDCT because the tumor depiction on CEUS is less affected by iodized oil retention. High signals caused by accumulated Lipiodol (can mask enhancement of residual tumor) makes it difficult to evaluate treatment response and constitute the most important disadvantage that affects MDCT. The three false-negative cases found at MDCT showed a complete homogeneous retention of iodized oil.

A specific limitation of this study lies in the use of an imperfect reference standard (imperfect reference bias). The reference-standard procedures (MDCT in case of RFA treatment and angiography in case of TACE treatment) yield results that are not nearly 100% accurate in detecting recurrent HCC. When compared with histopathologic findings, MDCT sensitivity and specificity are, respectively, 36% and 100% [21], whereas angiography shows diagnostic efficacy in detecting recurrent HCC after TACE of 47% [22]. We think, however, that MDCT and angiography yields results that are consistent with clinical objective (response to treatment).

## Conclusion

In summary, we found that CEUS enables radiologists to confidently assess the therapeutic effect of nonsurgical treatment on HCC.

We highly recommend assessing residual intratumoral flow on CEUS during RFA procedure to determine the necessity of immediate additional treatment. This approach should reduce the costs and allow achieving optimal patients' management and treatment results.

In our opinion, CEUS could be considered the exam of first choice in monitoring the efficacy of TACE. We think that, in the case of CEUS viable tumor findings, the HCC nodule treated with TACE should be considered still viable and undergo an additional treatment without further CT assessment.

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