

## Contrast-Enhanced Ultrasonography: A Recent Application for the Diagnosis and Treatment of Hepatocellular Carcinoma

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### ABSTRACT

Hepatocellular carcinoma has a decisive influence on the prognosis of cirrhotic patients, and the use of imaging modalities is essential for the screening, diagnosis and treatment of hepatocellular carcinoma. Ultrasound plays a major role among them, because it provides real-time and non-invasive observation by a simple and easy technique. In addition, ultrasound-guided needle puncture methods are frequently required for the diagnosis and/or treatment process of hepatocellular carcinoma. The development of digital technology has led to the detectability of blood flow by color Doppler ultrasound, and the sensitivity for tumor vascularity has shown remarkable improvement with the introduction of microbubble contrast agents. Moreover, near real-time three-dimensional ultrasound images are now available. These advancements in the ultrasound field have led to rapid progress in hepatocellular carcinoma management, and continuing advances are expected. This article reviews the current application of contrast-enhanced ultrasound for hepatocellular carcinoma in clinical practice.

**Key words:** *contrast agent, hepatocellular carcinoma, ultrasound*

### INTRODUCTION

Hepatocellular carcinoma (HCC) is increasing worldwide and is one of the most common carcinomas in the eastern part of Asia.<sup>1-2</sup> As the prognosis of cirrhotic patients depends on the occurrence and progression of HCC, diagnosis and treatment of this neoplasm are major issues in clinical practice.

Recent advances in digital technologies have resulted in remarkable developments in the field of imaging modalities. Ultrasound (US) is one of the diagnostic tools that have shown significant improvement within the last decade.<sup>3</sup> As for the diagnosis of liver tumors,

US examination has the advantages of real-time observation, simple technique and non-invasiveness. It is being used worldwide, and at a high frequency, as a first-step, reliable method for the diagnosis of liver tumors. Further, the application of microbubble contrast agents provides details of the hemodynamics, which are useful for the detection and characterization of liver tumors.<sup>4-5</sup> Diagnosis and treatment of HCC have shown remarkable improvement with the clinical usage of contrast-enhanced US.

This article reviews the recent application of contrast-enhanced US for the management of HCC.

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## Color Doppler US

The advent of the Doppler method has turned around diagnostic ultrasound.<sup>3-4,10-12</sup> The waveform analysis was the primary application, and it was frequently used for the characterization of liver tumors. Then, color flow imaging with real-time observation added to the diagnostic process of liver tumors, and power Doppler mode contributed to a better detectability of blood flow.<sup>13-21</sup> However, limitations in the detection of slow flow and vessels deeply located from the skin surface have prevented the wider application of Doppler mode in the evaluation of tumor hemodynamics. Furthermore, artifacts caused by respiratory or cardiac motion sometimes affect the precise evaluation of hemodynamic information. At present, the application of Doppler mode alone for detecting tumor blood flow is rare, as the more recent availability of microbubble contrast agents has assisted in overcoming those limits.

## Microbubble contrast agents

With the above as a background, there has been considerable expectation that US contrast agents would improve the detectability of blood flow in liver tumors. In fact, since the first report about a US contrast agent by Gramiak et al. There has been an ongoing challenge to establish a contrast-enhanced US with microbubble agents.<sup>22-25</sup> While free gas bubbles are efficient scatterers of ultrasound, their utility has been limited because of their immediate removal by the lungs. From the late 1980s to the 1990s, grey-scale contrast-enhanced US with carbon dioxide gained broad attention as an echo-enhancing technique, with high sensitivity for detecting tumor vascularity and high performance for the characterization of liver tumors.<sup>26-27</sup> However, the method requires an arteriography procedure because carbon dioxide is easily soluble in blood. The development of microbubble contrast agents available with peripheral venous injection was expected for practical use.

At the end of the 20th century, finally a galactose-based US contrast agent (SHU 508, Levovist) was made available by Schering, Germany. It was a long-awaited material that could provide a stable enhancement effect in abdominal organs with a peripheral injection. Subsequently, many microbubble contrast agents have been developed or are currently under development (Table 1). These intravenously injectable agents improve the detectability of tumor blood flow and the depiction of characteristic flow patterns in Doppler mode or contrast-specific imaging mode with less safety concerns.

## Characteristic property of microbubble contrast agents

### 1. Acoustic properties of microbubble and specific imaging mode

The microbubble agents have characteristic acoustic properties that depend on the size and kind of gas and shell.<sup>5,28</sup> Contrast harmonic imaging mode, which is based on non-linear scattering behaviors of microbubbles, provides high resolution with fewer artifacts compared to the simple Doppler technique, and is now a representative imaging method specialized for microbubble contrast agents.<sup>29-30</sup>

The behaviors of microbubbles are related to the acoustic power level (MI; Mechanical Index) of the transmitted ultrasound. As for Levovist, US transmission under a standard acoustic power level for routine US examination provides immediate disappearance of the microbubbles. This feature, "loss of correlation" ("stimulated acoustic emission"), is used in some imaging modes specialized for Levovist.<sup>31-32</sup> Additionally, the echo signal increases according to lengthening of the interval between frames, as the intermission during US transmission reduces the destruction of microbubbles in the region of interest (ROI) and help microbubble to gather in the US field. With this methodology, an ingenious contrivance of intermittent transmission technique has been developed to obtain strong echo-enhancement in the ROI as a contrast-specific imaging.<sup>33</sup>

The next-generation contrast agents, such as SonoVue (Bracco Diagnostics, Princeton, NJ, USA) and Definity (Bristol-Myers Squibb, N. Billerica, MA, USA), have characteristic oscillation behavior under very low MI which provides the fewer tissue signals and less microbubble breakdown. As a result, contrast harmonic imaging under low MI level has received considerable attention of late as a useful method for real-time observation of microbubble images, and it is expected to improve the diagnostic ability of liver tumors (Fig. 1).<sup>34-38</sup>

### 2. Accumulation property of microbubble

The diagnostic performance of contrast-enhanced US is not limited to the demonstration of tumor vascularity. Some microbubble contrast agents have a characteristic property of organ-specific accumulation.<sup>36-39</sup> Although the precise mechanism remains unclear, the reticuloendothelial system (i.e. phagocytosis by Kupffer cells) may be involved in this phenomenon. Both Levovist and Sonazoid (GE Healthcare, Oslo, Norway) accumulate in the liver, and sonograms in this phase (late liver-specific parenchymal phase) are frequently used for the

detection or characterization of liver tumors. In contrast, Definity and SonoVue do not accumulate in the liver. The characterization of liver tumors by contrast-enhanced US has been carried out using accumulation images as well as vascular enhancement images (Figure 2A, B).

## Clinical application of contrast-enhanced US

### 1. Detection of vascularity in HCC

Microbubble contrast agents can increase the detectability of blood flow by US examination. Numata et al. reported that the same results in detecting tumor vascularity, 53/61 nodules (87%), between contrast-enhanced harmonic grey-scale imaging with Levovist and helical CT.<sup>39</sup> More recent studies also showed over 80% concordance of tumor vascularity (Giorgio et al., 82.4%; Bolondi et al., 81%) between contrast-enhanced US under low MI level with SonoVue, and contrast-enhanced helical CT.<sup>40-41</sup> The improved detectability of tumor vascularity in contrast-enhanced US contributes to the characterization of liver tumors and assessment of the therapeutic response.

### 2. Characterization of focal liver lesions

Many studies using contrast-enhanced US have been carried out for the characterization of focal liver lesions with early-phase images and/or delayed phase (liver-specific phase) images (Figure 1, Table 2). Early-phase images provide characteristic vascular-enhancement patterns that are useful for specific diagnosis.<sup>42-44</sup> However, evaluation of the enhanced appearance in delayed-phase images is not always simple owing to the fact that the accumulation property of microbubbles affects the enhancement findings in this phase. According to previous reports, focal nodular hyperplasia (FNH) shows positive enhancement and metastatic tumor shows negative enhancement in the delayed phase with Levovist, a contrast agent with accumulation property in the liver.<sup>45-48</sup> However, it is known that several enhancement patterns are observed in both HCC and hepatic hemangioma in delayed phase sonograms.<sup>45-48</sup> Despite the various kinds of contrast agents, evaluation of combined multi-phase images would improve the diagnostic ability of contrast-enhanced US.

Concerning the discrimination of malignant versus benign liver lesions by contrast-enhanced US, recent literatures have reported sensitivity of 98 to 100% and specificity of 63 to 93% with Levovist, and sensitivity of 98% and accuracy of 92.7% with SonoVue.<sup>45-50</sup> Furthermore, in a clinical study with two independent image reviewers, Kim et

al. described that contrast-enhanced US (agent detecting imaging mode with Levovist) provided a specific diagnosis in 75-79% of 75 patients with focal hepatic lesions, and that the technique was successful as a confirmatory imaging technique in 63-72% of the patients.<sup>51</sup>

Small HCC nodules less than 20 mm sometimes present a hypovascular appearance by imaging modalities, and both dysplastic nodules and regenerative nodules also appear as hypovascular nodules.<sup>41,52-54</sup> Since high-grade dysplastic nodules are considered as potentially pre-malignant lesions, the characterization of such hypovascular nodules is very important in clinical practice.<sup>55-56</sup> The recent study reported that intensity analysis of contrast enhancement with Levovist was useful method for the characterization of non-hypervascular small hepatic nodules, regenerative nodule from HCC.<sup>57</sup> However, the diagnostic ability of contrast-enhanced US in the early diagnosis of HCC and discrimination of dysplastic nodules from HCC has not been established. At present, percutaneous needle biopsy under US-guidance may be frequently required for the characterization of small hepatic lesions in patients with chronic liver diseases.

Hypervascular hepatic lesions do not always reflect the fact that the final diagnosis of the nodule is HCC in heavy drinkers, since benign hypervascular nodules sometimes occur in their liver.<sup>58</sup> A recent report has shown that the ring-shaped appearance on liver-specific contrast-enhanced sonograms with Levovist may be a useful sign for the differential diagnosis of benign nodule from HCC in heavy drinkers.<sup>59</sup> Since contrast-enhanced CT hardly differentiates these benign nodules from HCC, this characteristic finding may prevent unnecessary treatments under misdiagnosis. Moreover, it could be expected to lead to a reduction in the application of percutaneous needle biopsy, an invasive procedure, for the precise diagnosis.

### 3. Detection of tumor nodules in the liver

Some kinds of hepatic nodules, such as metastatic liver tumors, appear as hypo-enhanced nodules on liver-specific images by accumulated microbubbles in the liver. Since grey-scale US sometimes fails to detect metastatic tumor nodules because of their small size and iso-echoic appearance, contrast-enhanced liver-specific sonograms assist in detecting occult tumor nodules on grey-scale images.<sup>30,60-64</sup> This application of US contrast agents contributes to the staging of the disease by a non-invasive procedure.

Meanwhile, as neither Definity nor Sonovue accumulate in the liver, they do not provide liver-specific phase which is useful to detect occult tumor nodules (blood-pool contrast agents). However, previous study showed that contrast-enhanced US with Definity improved the detection rate of hepatic tumors in rabbit liver.<sup>65</sup> As a suitable MI for this agent is quite low level which allows less microbubble breakdown, repeated observation for contrast-enhancement is possible. However, comparison of detectability of tumor nodules between the agent with accumulation property and the agent without accumulation property remains to be solved.

Recent US systems have provided three-dimensional visualization of the tumor with tumor-associated vessels at any plane from multiple directions.<sup>66-69</sup> Contrast-enhanced 3D ultrasonographies using microbubble contrast agents might become a standard method for the characterization of hepatic tumors (Figure 2).

## Treatment support and evaluation of therapeutic effect

### Treatment of HCC

#### 1. Percutaneous needle puncture technique

As the majority of patients with HCC have liver dysfunction, surgery is not always an appropriate treatment choice.<sup>2,70-71</sup> In addition, recurrence of HCC is an inseparable companion of post-treatment patients. With such backgrounds, percutaneous ethanol injection (PEI) and radio-frequency ablation (RFA) were developed and came to be widely used in clinical practice as minimally invasive methods.<sup>72-77</sup> They are now a first-line, favored approach that has an efficient therapeutic effect on HCC.<sup>78-84</sup>

#### 2. Problem for US-guided treatments

Although percutaneous US-guided treatments provide sufficient therapeutic effect, recurrence often plagues many HCC patients. According to long-term study results, cumulative recurrence rates of the treated site of post-PEI lesions were 3.4% at 1 year, 7.1% at 2 years, and 10% at 3 years, and those of the untreated sites in liver were 18.7% at 1 year, 62.1% at 3 years, and 81.7% at 5 years, respectively.<sup>80</sup> Thus, many HCC patients have to receive repeated treatments during their clinical course. In order to minimize adverse effects to the liver, less invasive treatment such as PEI or RFA is preferable for these patients. However, localization of lesions on the sonograms is sometimes problematic in patients with cirrhotic liver and/or repeated treatment history (Figure 3).<sup>85-86</sup> Although percutaneous treatment under CT guidance is a well-established technique and a useful

method for lesions undetected by US, the method lacks convenience and exposes both patients and physicians to radiation.<sup>87-90</sup> Microbubble contrast agents are also useful in such a case. A recent study showed that contrast-enhanced US with Levovist could localize 24/32 (75%) of HCC lesions that were invisible by non-contrast US.<sup>91</sup> Application of the next-generation US contrast agents is expected to improve the ability for tumor localization, because they have acquired stability of microbubble homogenization of particle size distribution in comparison with earlier agents.<sup>5,92</sup> Moreover, the combination of second-generation contrast agents with harmonic imaging mode under lower MI may produce US images with improved signal-to-noise ratio, and a higher detection rate of focal lesions in the liver is expected.<sup>36,49</sup> The detectability of ultrasonically unrecognizable hypervascular HCC with Sonazoid, a newly developed perflubutane agent, was reported to be 96.4%, which was quite higher than that with Levovist.<sup>93</sup> Contrast-enhanced US with Sonazoid would widen the application of percutaneous US-guided treatments (Figure 4).

#### 3. Assessment of therapeutic response by ultrasound

With high sensitivity and specificity for detecting tumor vascularity, contrast-enhanced US has come to be frequently applied for evaluation of the therapeutic response in HCC nodules (Table 3). As for percutaneous treatment, Bartolozzi et al. reported that contrast-enhanced color Doppler with Levovist showed sensitivity of 92%, specificity of 100%, and accuracy of 98% compared to the results of spiral CT and biopsy, in the detection of viable tumor treated with PEI.<sup>94</sup> A study by Choi et al. revealed that the diagnostic agreement between contrast-enhanced power Doppler with Levovist 14-23 hours after ablation therapy and immediate follow-up CT within 30 minutes after ablation therapy was achieved in 100% of the 45 HCC nodules.<sup>95</sup> In an assessment of therapeutic response after RFA, Wen et al. compared the result of contrast-enhanced coded harmonic angio mode with Levovist for detecting residual tumor in 91 HCC nodules 5 to 7 days after RFA with that of dynamic CT, and the sensitivity, specificity, and diagnostic accuracy were 95.3%, 100%, and 98.1%, respectively.<sup>96</sup> According to the study by Meloni et al., the sensitivity and specificity of contrast-enhanced harmonic US with Levovist were 83.3% and 100%, respectively, for detecting residual non-ablated tumor at 4 months after treatment in 43 HCC nodules, compared with helical CT findings.<sup>97</sup>

As for assessment of the therapeutic response after transarterial chemoembolization (TACE), it is well known that contrast-enhanced US has the advantage of not being limited by iodized oil

**Table 1. US contrast agents (quotation from World J Gastroenterol 2008;21:1710-9)**

	Gas	Shell	Diameter*	Manufacturer
Al-700	Perfluorocarbon	Synthetic polymer	?	Acusphere
Albunex	Air	Cross-linked albumin	3-5 (4.3)	Molecular Biosystems
iosphere	Air	Biodegradable polymer	?	Ponit Biomedical
Definity	perfluoropropane	Lipid	2.3	Bristol-Myers Squibb (DuPont Merck †)
Echovist	Air	Galactose	3	Schering
Echo Gen	Dodecafluoropentane	Surfactant	0.4, 2-5 ‡	Sonus Pharmaceuticals
Imavist	Perfluorohexane Physiological gases	Surfactant	<5	Alliance Pharmaceutical
Levovist	Air	Galactose Palmitic acid	1.3	Schering
Optison	Perfluoropropane	Cross-linked albumin	3.6-5.4 (4)	Molecular Biosystems
Sonavist	Air	Cyanoacrylate(polymer)	1	Schering
Sonazoid	Perfluorocarbon	Lipid	2.4-2.5	Nycomed-Amersham
SonoVue	Sulfur Hexafluoride	Phospholipids	2.5	Bracco Diagnostics
uantison	Air	Albumin	3.2	Quadrant

\*: Diameter of microbubble ( $\mu\text{m}$ ). Numbers in parentheses are mean diameters.

†: Prior manufacturer

‡: Echo Gen is the first phase shift US contrast agent which has a boiling point significantly below body temperature. It has two different conditions, a liquid at room temperature (non echogenic particles with a mean diameter of approximately  $0.4\mu\text{m}$ ) and a gas at body temperature (echogenic microbubbles with a diameter of  $2-5\mu\text{m}$ )

**Table 2. Characterization of focal hepatic lesions by contrast-enhanced US**

Author	Contrast agent	No. of patients	Results*
Bryant TH, et al. [47]	Levovist	88	Sensitivity§ 89, 93%
		54	Specificity§ 80, 93%
		142	Accuracy§ 88, 90%
Dietrich CF, et al. [48]	Levovist	174	Sensitivity 100% Specificity 93%
Kim SH, et al. [51]	Levovist	75	Sensitivity§ 98, 98%
			Specificity§ 85, 91%
			SD†, § 75, 79%
			CIT‡, § 63, 72%
von Herbay A, et al.[45]	Levovist	67	Sensitivity 100%
			Specificity 63%
Nicolau C, et al. [50]	SonoVue	152	Sensitivity 98%
			Specificity 82%
			Accuracy 92.8%

\*: Discrimination of benign and malignant lesions

†: Specific diagnosis: Correct diagnosis was obtained by contrast-enhanced US

‡: Confirmatory imaging technique: The reader judged that no further imaging for lesion characterization was needed and that the lesion concerned had been correctly diagnosed.

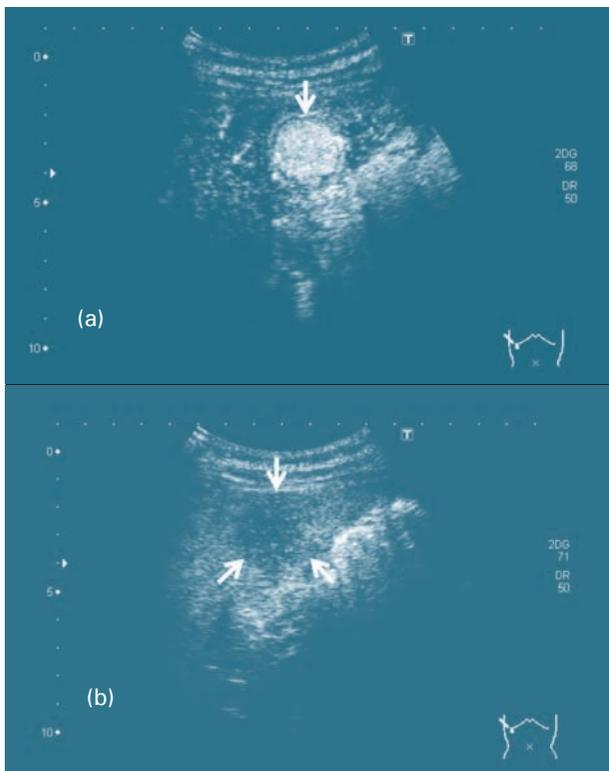
§: Evaluation by two different readers

**Table 3. Assessment of therapeutic response after radiofrequency ablation (RFA) for HCC using contrast-enhanced US**

Author	No. of patients/ No. of lesions	Results*
		(contrast agent)
Wen et al [96]	67/91	Sensitivity 95.3% Specificity 100% Accuracy 98.1% (Levovist)
Meloni et al [97]	25/43	Sensitivity 83.3% Specificity 100%(Levovist)
Choi et al [95]	40/45	Diagnostic agreement 100%(Levovist)
Kim et al [101]	90/94	Diagnostic concordant † 99% (Levovist)
Solbiati et al [102]	20/20	Sensitivity 50% Specificity 100% Diagnostic agreement 85%(Levovist)

\*: Comparison with contrast-enhanced helical CT

†: 1-month follow-up CT

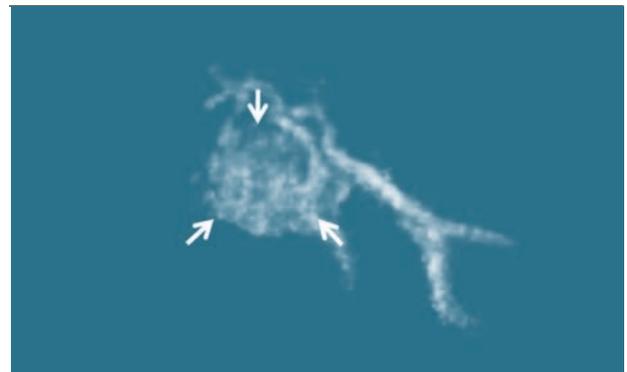


**Figure 1.** Contrast-enhanced harmonic imaging with Sonazoid in HCC (26.3 mm, arrows)

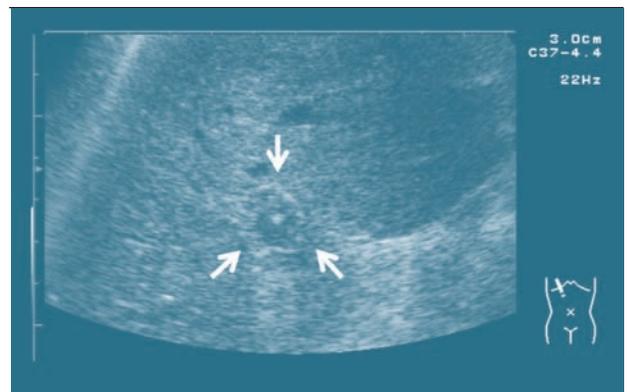
**a)** Early-phase image (27 seconds after the injection)

**b)** Late-phase image (10 minutes after the injection)

The early-phase image showed positive enhancement and the late-phase image showed negative enhancement in the nodule. These findings provided easy diagnosis of HCC.



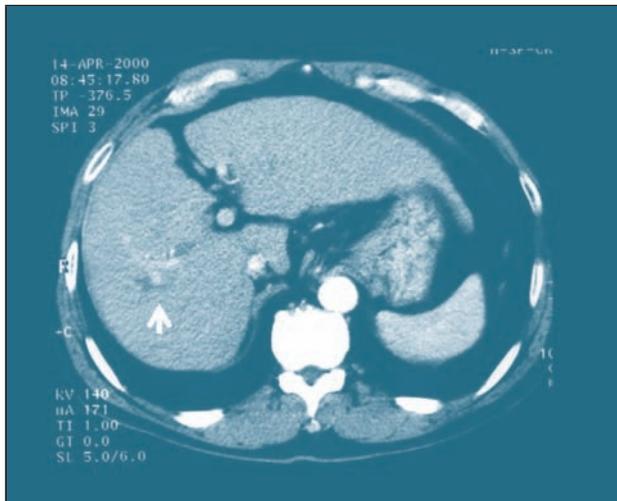
**Figure 2.** Three-dimensional ultrasonography (contrast-enhanced 3DUS with Sonazoid, arrows) showed hypervascular tumor with abundant fine tumor vessels.



**Figure 3 (a).** HCC (S8, 29.4 mm), local recurrence of HCC after PEI B-mode US

Treated area of HCC after PEI (arrows)

There was no identification whether the tumor was viable or not.



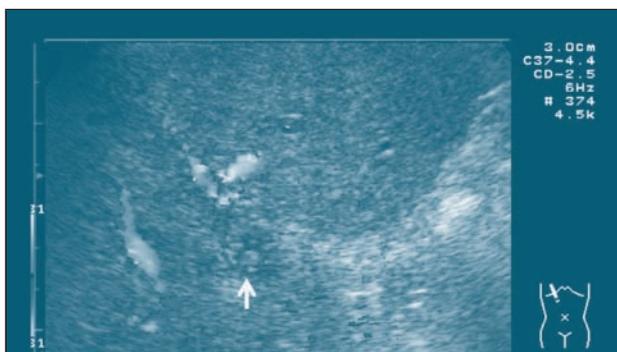
**3 (b). Contrast-enhanced CT**

Contrast enhancement at the local recurrence lesion was observed in the treated area (arrow).



**3 (c). Color Doppler US**

No findings appeared in the treated area on the image (arrows).



**3 (d). Contrast-enhanced US with Levovist**

Contrast-enhanced color Doppler could demonstrate a blood flow which was similar to contrast-enhanced CT finding (arrow).



**3 (e). Contrast-enhanced US with Levovist after PEI**

Color flow signal disappeared after the treatment.



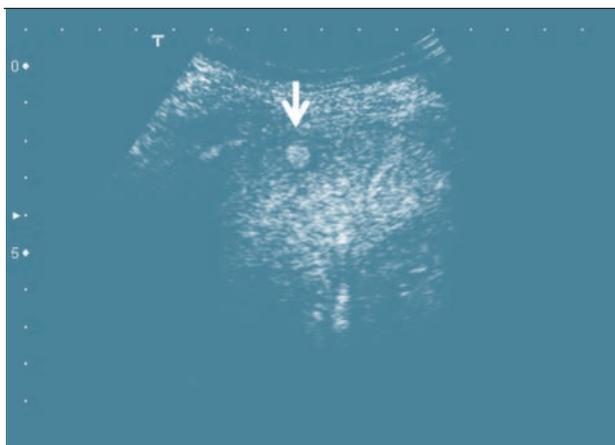
**Figure 4 (a). Detection of ultrasonically invisible hypervascular HCC using contrast-enhanced US with Sonazoid a) Contrast-enhanced CT**

Hypervascular lesion which was local recurrence of HCC was observed on contrast-enhanced CT image (arrow).



**4 (b). B-mode US**

Hepatic lesion was not recognized on B-mode sonogram.



**4 (c). Contrast-enhanced US with Sonazoid**

Hepatic lesion which corresponded to CT finding was demonstrated on contrast-enhanced US image with Sonazoid (arrow).

deposition that affects the evaluation of contrast-enhanced CT findings. Minami et al. reported that contrast-enhanced harmonic US with Levovist had a significantly higher sensitivity in depicting residual blood flow in HCC about one week after TACE than dynamic CT and dynamic MR imaging: contrast-enhanced US: 38 of 44 (86%) lesions, dynamic CT: 19 of 44 (43%) lesions, dynamic MR imaging: 10 of 20 (50%) lesions.<sup>98</sup> They also added that the contrast-enhanced US findings are predictive for local recurrence of HCC in the clinical course after

treatment. Morimoto et al. compared the results of contrast-enhanced US with histologic findings, and sensitivity and specificity for discerning viable and nonviable HCC after TACE in 29 HCC nodules were 100% and 81%, respectively.<sup>99</sup> With the use of SonoVue, Pompili et al. described that contrast-enhanced US resulted in diagnostic agreement in 53/56 cases (94.6%), with sensitivity and specificity of 87.0% and 98.4%, respectively, after non-surgical treatments for HCC (PEI, RFA, TACE, TACE followed by PEI, RFA followed by PEI), compared with contrast-enhanced CT findings.<sup>100</sup>

As mentioned above, the diagnostic ability with the assessment of therapeutic response in HCC is now sufficient, and is equivalent to contrast-enhanced CT findings. Although there are some limitations that the performance of US examination depends on the operator's skill, location of the tumor and system capability, contrast-enhanced US would play a major role in evaluation of the therapeutic effect of HCC. The recent developments in this technology would allow contrast-enhanced US to be positioned as the standard method for evaluation of the therapeutic effect in many HCC patients.

In conclusion, US has made amazing strides in the last decades because of digital technology progress, and it will continue to grow. The advancement of imaging methods is expected to support the clinical management of patients with HCC.

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