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Quantitative comparison of tumor vascularity of hepatocellular carcinoma after intravenous contrast agent: conventional versus harmonic power Doppler US

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Abstract

Background: The purpose of this study was to make a quantitative comparison between conventional and harmonic power Doppler (PD) ultrasound (US) in depicting vascularity of hepatocellular carcinoma (HCC).

Methods: Ten nodular HCCs in 10 patients were prospectively examined using a 2–4-MHz convex transducer and a standardized examination protocol. Serial US images were obtained before and 20, 30, 40, 50, 60, 90, 120, 150, 180, 240, and 300 s after intravenous injection of 2 g of contrast agent using conventional and harmonic PD US. The percentage of area with Doppler signal within each HCC nodule (%PDA) was calculated in each image with a PC-based image analysis program, and the results with both US techniques were compared.

Results: In the majority of cases, %PDA was greater on conventional PD US than on harmonic PD US. Mean %PDA of 10 HCCs was significantly higher on conventional PD US than on harmonic PD US except at 20 s after injection. The highest values of mean %PDA were 34.9% in conventional PD US and 19.5% in harmonic PD US at 60 s after injection.

Conclusion: Area with PD signals within the HCC is smaller and the duration of effective enhancement is shorter in harmonic PD US than in conventional PD US.

Key words: Liver neoplasm, US—Ultrasound, contrast media—Ultrasound, power Doppler studies—Ultrasound, comparative studies.

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Power Doppler (PD) ultrasound (US) is a technique that displays the total integrated power of the Doppler signal. PD US has been reported to be more sensitive than color Doppler US in the demonstration of vascular flow within hepatic tumors including hepatocellular carcinoma (HCC) [1, 2]. Many US contrast agents using different gases and coatings have recently been developed and used in the field of medical US. The US contrast agent is thought to improve the performance of Doppler study, and several recent studies have reported that PD US using a US contrast agent showed more tumor vascularity in hepatic tumors than did unenhanced PD US [3, 4].

Harmonics are frequencies that occur at multiples of the fundamental or transmitted frequency. Second harmonic US technique transmits at frequency f and receives at frequency $2f$. Several studies have suggested that harmonic US may improve signal-to-noise ratio, contrast-to-noise ratio, and spatial resolution [5–7]. In Doppler US, harmonic US is also thought to decrease flash artifacts of PD US and blooming artifacts associated with US contrast agent [8–10]. Therefore, harmonic PD US using US contrast agent may provide high-quality information about vascular flow in various lesions.

We performed this prospective study to compare the dynamic enhancement in nodular HCC on contrast-enhanced conventional and harmonic PD US. Many studies on the in vivo evaluation of PD signal were principally subjective and used descriptive criteria for semiquantitative estimation of Doppler signal [3, 4]. In this study we designed a method for the quantification of PD signal and performed a quantitative comparison of the two contrast-enhanced PD US techniques.

Materials and methods

Subjects

During a 5-week period, 43 patients with newly detected HCCs were referred to our department for transcatheter arterial chemoembolization (TACE). Among them, 12 patients who had single or multiple nodular HCCs not treated previously were prospectively studied with contrast-enhanced conventional and harmonic PD US. Thirty-one other patients who had massive or diffuse type of HCC were excluded from the study. Two of 12 patients undergoing contrast-enhanced conventional and harmonic PD US were excluded because of motion artifacts in conventional PD US, which disabled quantitative measurement of the PD signal. Thus, the resultant study population consisted of 10 patients (nine male, one female; age range = 44–66 years, mean = 54 years). The diagnosis was confirmed by means of percutaneous needle biopsy in five patients and by clinical and laboratory data (positive for HBsAg or anti-HCV and serum α-fetoprotein level above 100 ng/mL) and typical angiographic findings in the others. Three patients had multiple nodules of HCC. In those patients, we selected only the largest one that was well visualized on US because serial dynamic US scanning was possible for only one lesion in each patient. The longest dimension of tumors as measured on sonograms ranged from 2.3 to 6.5 cm (mean = 3.9 cm). Nine lesions were in the right lobe and one lesion was in the left lobe.

US examination

US scanning was performed by one radiologist (T.K.K.) using a HDI 3000 unit (Advanced Technology Laboratories, Bothell, WA, USA) and a 2–4-MHz curved-array probe. US scanning protocols were as follows: pulse repetition frequency (PRF) of 1000 Hz and medium wall filter (high pass filter) for conventional PD US and PRF of 700 Hz and low wall filter for harmonic PD US. The color-write priority was set at the maximum. In each case, the color gain was set at maximal level to eliminate color noise in the background image of PD US: The color gain was increased enough to cause color noise and then the color gain was slowly decreased until the color noise disappeared. The resultant color gains used in the examination of HCCs ranged 68% from 84% for conventional PD US and from 90% to 100% for harmonic PD US. The US contrast agent SH U 508A (Levovist; Schering AG, Berlin, Germany) was prepared as follows. Before the US examination, 4 g of this agent was prepared by shaking for 5–10 s with 11 mL of sterile water. About 13 mL of milky suspension of galactose microparticles and micro–air bubbles with a concentration of 300 mg/mL was created by disaggregation of the granules. After standing for 2 min for equilibration, for each examination, 6.5 mL (2 g) of the contrast agent suspension was injected manually through a 20–22-gauge cannula placed in an antecubital vein followed by an additional 10 mL of physiologic saline to flush the cannula. Injection rate was approximately 0.2 mL/s for conventional PD US and 0.6 mL/s for harmonic PD US. Serial static US images were captured using conventional PD US before and 20, 30, 40, 50, 60, 90, 120, 150, 180, 240, and 300 s after intravenous injection of 6.5 mL of contrast agent suspension. The scan plane was determined to include the center of each lesion, to visualize the lesion clearly, and to minimize artifacts from the patient's motion. Each static image was obtained during suspended respiration. Five minutes after first imaging, the same procedure was repeated using harmonic PD US at the same scan plane as conventional PD US.

By preliminary scanning of a few patients before the study, we found that slow injection of contrast material and PD settings of low or medium sensitivity to flow could not produce sufficient enhancement of the liver on harmonic PD US. In conventional PD US, lowering PRF and wall filter was not helpful to detect slow vascular flow because PD artifacts also markedly increased, and these artifacts could obscure or mimic true vascular flow. In harmonic PD mode, use of PRF of 700 Hz and low wall filter produced only minimal PD artifacts. For this reason, we used different protocols for PD scanning and contrast injection between both imaging techniques. In the present study we used variable color gains in each examination. Keeping the color gain constant may be more appropriate for strict quantitative comparison of the two US techniques. However, a color gain level that was appropriate for one case could create much noise in other cases, making measurement of Doppler signal impossible. For this reason, we used variable color gains in each examination. From our experience, maximal color gain to eliminate obscuration of the image due to color noise is preferred for sensitive detection of vascular flow in various lesions. Therefore, we used maximal color gain level to eliminate color noise in the background image.

Image analysis

For the quantification of PD signal we calculated the percentage of area with PD signal within each HCC nodule to the total area of the nodule (%PDA) using a PC-based program that we designed. The color of a pixel in a digital image is determined by the combination of three primary colors: red (R), green (G), and blue (B). In 24-bit RGB bitmap images, 8 bits are assigned to each primary color, and each primary color can have 256 values (0–255) representing their intensity. Therefore, by combining three primary colors, more than a million colors ($256 \times 256 \times 256$) can be created. Zero for all of R, G, and B produces black and 255 for all of R, G, and

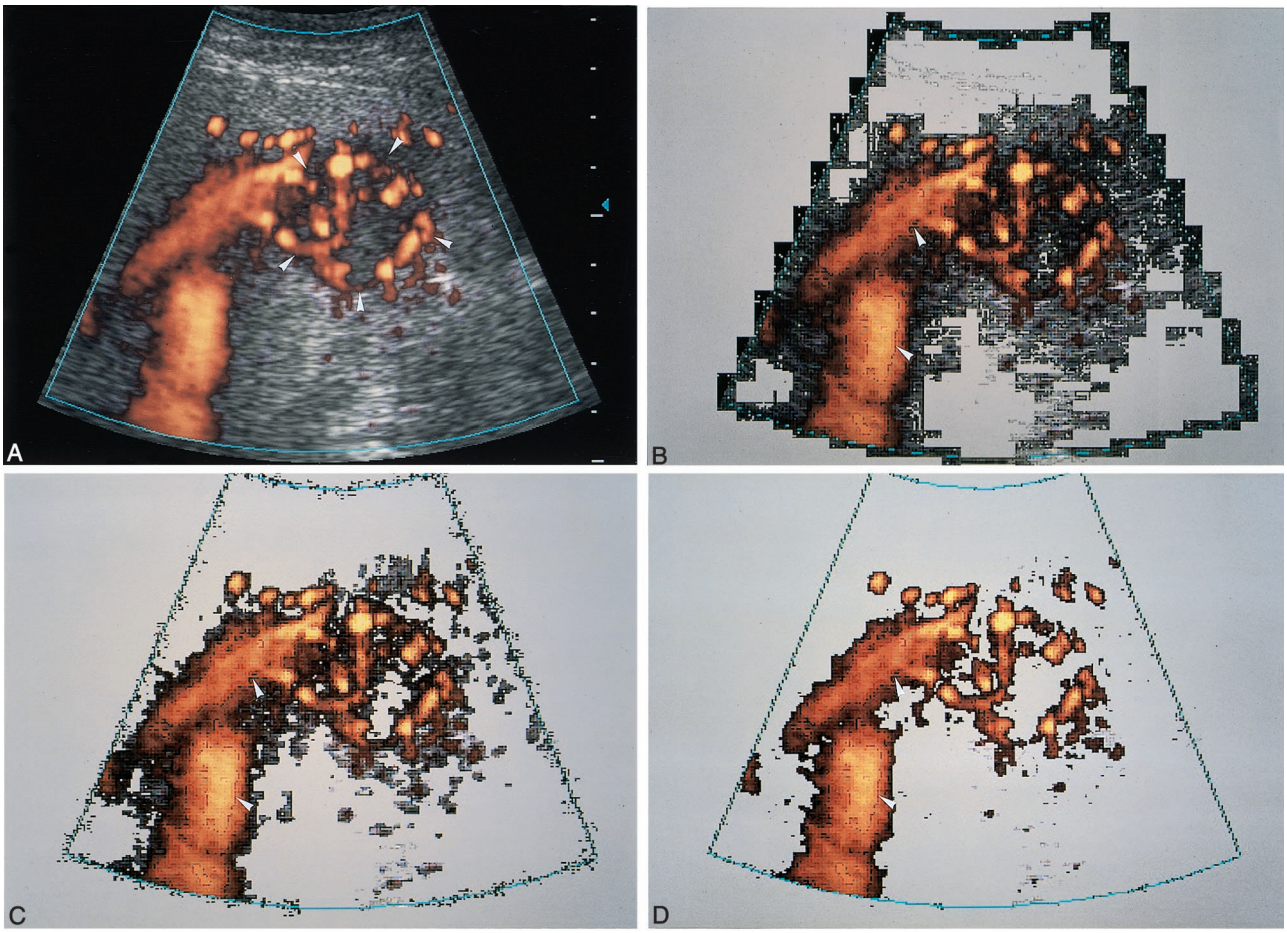


Fig. 1. Separation of area with PD signal from the background gray-scale image. **A** Original conventional PD US image of a 3-cm HCC nodule (*arrowheads*) obtained 40 s after contrast injection. **B** Pixels where at least R, G, or B has value different than the others: $R \neq G$ or $G \neq B$, or $R \neq B$. **C** Pixels where at least R, G, or B has a value of at least 15 greater than the others: $|R - G| \geq 15$, $|G - B| \geq 15$, or $|R - B| \geq 15$. **D** Pixels where at least R, G, or B has a value of at least 30 greater than the others: $|R - G| \geq 30$, $|G - B| \geq 30$, or $|R - B| \geq 30$. As the threshold of difference between the values of R, G, and B of a pixel increases, vascular flow signal becomes more clearly separated from the background image. Because **B–D** are screen-captured and magnified images, some pixels (*arrowheads* in **B–D**) appear to have colors not present in the original PD US image (**A**).

B produces white. Gray-scale pixels are those having the same values of R, G, and B, whereas color pixels are those with at least one color component whose value is different than the others. Therefore, with R, G, and B values for each pixel, color pixels can be separated from gray-scale pixels.

In a standard PD image, the PD signal is mapped with color pixels and the background image is mapped with gray-scale pixels. Therefore, areas with PD signal can be separated from the background gray-scale image with the same principle. Theoretically, all pixels, where at least R, G, or B has value different than the others, should be classified into color pixels and regarded as the PD signal. However, with such strict criterion, more pixels than we expected were selected, and some of the selected pixels were present where color pixels should have been absent or were thought not to represent true vascular flow (Fig. 1). By empirically increasing the minimal difference between values of R, G, and B needed for a certain pixel to be selected as true vascular flow signal, a more reasonable result could be achieved (Fig. 1). Therefore, we empirically set 30 as a threshold of difference between R, G, and B values of a pixel, and for each pixel in US images a value greater than the threshold was used to classify the pixel as vascular flow signal. The region of interest (ROI) was defined in each PD US image by carefully drawing a line along the tumor margin. The %PDA was then calculated with the following equation: $\%PDA = Nc/N \times 100$, where Nc is the number of pixels classified as vascular flow signal within ROI and N is the total number of the pixels within ROI.

In our study, the static US images were first stored in an HDI 3000 unit (Advanced Technology Laboratories, Bothell, WA, USA) in CRI format and then transferred to

an IBM-compatible PC using an optical disk. Images were then converted into 24-bit RGB bitmap files using a commercial software (ACDSee32; ACD Systems, Arlington, TX, USA). The 24-bit RGB bitmap files were used for image analysis by using a program designed with this principle. The %PDA of each HCC nodule at each scan time was obtained, and the results on conventional PD US and harmonic PD US were compared. Mean %PDA of 10 nodules of HCC was plotted against time after contrast injection, and the results on conventional PD US and harmonic PD US were compared. Statistical comparison between the two US techniques was performed with the Wilcoxon signed-rank test, and a p value of less than 0.05 indicated a statistically significant difference.

Results

Conventional PD US before contrast injection showed intratumoral PD signal in seven patients (70%) and their %PDA ranged from 2% to 19.4% (mean = 11.4%). In contrast, harmonic PD US before contrast injection depicted intratumoral PD signal in two patients (20%) and their %PDAs were 1% and 2.6%. After injection of the contrast agent, the amount of intratumoral PD signals increased in all cases. In all cases %PDA on conventional PD US was greater than those on harmonic PD US at almost all times (Fig. 2). The time to peak %PDA after contrast injection was variable and ranged from 30 to 90 s for conventional PD US and from 20 to 90 s for harmonic PD US. On conventional PD US, peak %PDA occurred at 30 s ($n = 2$), 40 s ($n = 1$), 50 s ($n = 2$), 60 s ($n = 3$), and 90 s ($n = 2$) after contrast injection (Table 1). On harmonic PD US, peak %PDA occurred at 20 s ($n = 1$), 40 s ($n = 3$), 50 s ($n = 1$), 60 s ($n = 3$), and 90 s ($n = 2$) after contrast injection (Table 1). In eight cases (80%), peak %PDA was greater on conventional PD US than on harmonic PD US (Table 1).

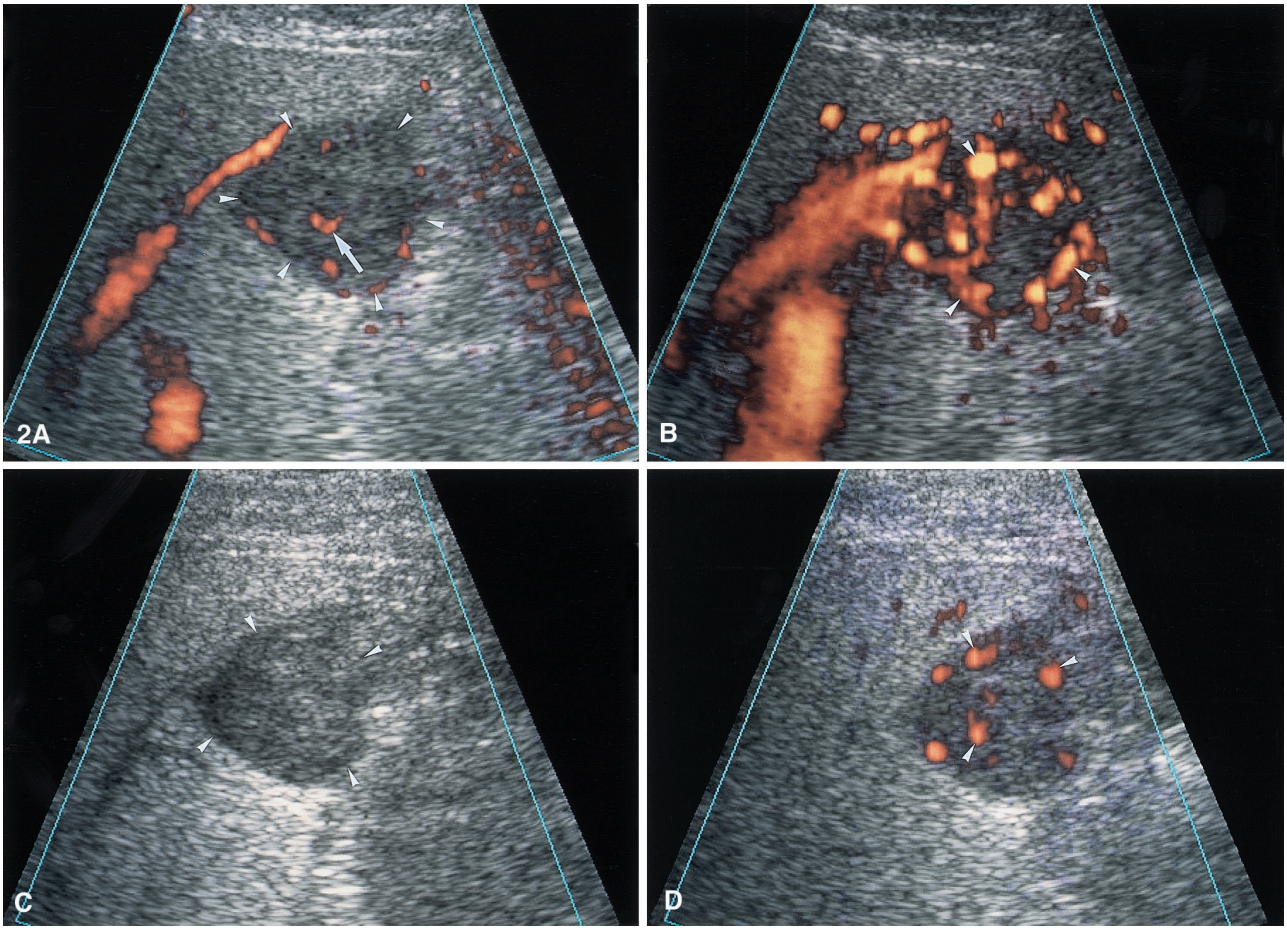
With both techniques, mean %PDA of 10 HCC nodules gradually increased, with the peak value at 60 s after contrast injection, and then gradually decreased (Table 2 Fig. 3). At 60 s after contrast injection, mean %PDA on conventional and harmonic PD US were 34.9% and 19.5%, respectively. At all times, mean %PDA on conventional PD US was greater than that on harmonic PD US and, except at 20 s, the two techniques showed a significant difference ($p < 0.05$; Table 2). On conventional PD US, mean %PDA remained high until 300 s, with 8.6% at 300 s (Table 2, Fig. 3). However, on harmonic PD US, although mean %PDA at 300 s was still higher than that of unenhanced state, mean %PDA decreased below 2.0% after 240 s (Table 2, Fig. 3).

Discussion

Harmonics are frequencies that occur at multiples of the fundamental or transmitted sonographic frequency. Second harmonic US transmits at frequency f and receives at frequency $2f$ by using a bandpass filter whose center frequency is at the second harmonic. Recent studies on the harmonic US have suggested that it may improve signal-to-noise ratio, contrast-to-noise ratio, and spatial resolution in medical US [5–7]. Because of the nonlinear property of microbubbles in the US field, harmonics are generated from microbubble contrast agents, a resonance phenomenon completely different from the mechanism of harmonic generation within tissue [6, 8, 10–12]. The magnitude of the backscattered signal from the microbubbles at harmonic frequency is much greater than the signal from the tissue at harmonic frequency. Therefore, when using harmonic US in concert with microbubble contrast agents, signals from the blood flow containing contrast agent can be received preferentially and signals from the tissue are reduced [10, 12, 13]. In Doppler US, harmonic US is also thought to decrease the image-degrading clutter or flash artifact and therefore may be an effective method to evaluate vascularity in patients with poor breath-holding ability or with lesions near the heart or great vessels [8–10].

In all cases in our study, %PDA on conventional PD US was greater than that on harmonic PD US at almost all times despite the higher color gain and more sensitive PD settings to flow in harmonic PD US as opposed to conventional PD US. This is not surprising considering that the returning harmonic signal is relatively weak compared with the signal at fundamental frequency [6]. Because blooming artifacts associated with US contrast agent are more prominent on conventional PD US than on harmonic PD US, we believe that the superiority of contrast-enhanced conventional PD US in demonstrating intratumoral vasculature of HCC over contrast-enhanced harmonic PD US is due in part to overestimation of vascular signals due to blooming artifact [8–10].

Several studies on the quantification of color Doppler (CD) or PD signal have been published [14–19]. Quantification of tumor vascularity with CD US was first demonstrated by an acetate-sheet overlay method [14, 15]. However, this semiquantitative and time-consuming method was impractical. In other studies, techniques for separating color components from gray-scale components in CD or PD US images using methods similar to ours have been suggested [16–19]. In our method, some of the pixels selected with a theoretically strict criterion for color pixels (all pixels where at least R, G, or B has a value different than the others) were present where color pixels should have been absent or were thought not to represent true vascular flow. Some of these values were probably due to electronic noise in the digitized image [16]. Although it is an assumption, other differences may



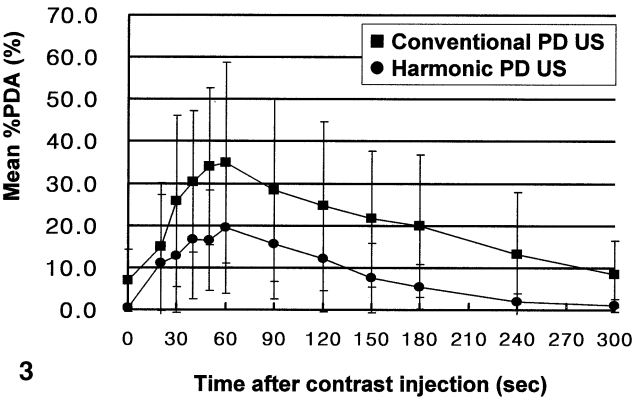


Fig. 2. Contrast-enhanced conventional and harmonic PD US of a 3-cm HCC nodule. **A** Conventional PD US image before contrast injection shows several dotlike and linear PD signals (*arrow*) within the hypoechoic tumor (*arrowheads*), with a %PDA of 6.6. **B** Conventional PD US image with peak enhancement obtained 40 s after contrast injection shows a marked increase of intratumoral PD signals (*arrowheads*), with a %PDA of 51.2. **C** Harmonic PD US image before contrast injection shows no intratumoral PD signals (*arrowheads*). There is also no detectable signal in the adjacent hepatic vessel. **D** Harmonic PD US image with peak enhancement obtained 50 s after contrast injection shows dotlike and linear PD signals (*arrowheads*) within the tumor, with a %PDA of 11. The intratumoral PD signal is greater on conventional PD US than on harmonic PD US.

Fig. 3. Mean %PDA of 10 HCC nodules on conventional and harmonic PD US plotted against time after contrast injection.

be related to minute changes of R, G, and B values during storage of US images in CRI format or during conversion of the CRI format into the 24-bit RGB bitmap format, producing some color pixels that are macroscopically indistinguishable from gray pixels in the interface between true flow signal and gray pixels or adjacent to the green line that defines the color box. Our method has the limitation of empirically solving this problem by setting a threshold to classify a pixel as colored with flow signal, and we could not directly assess the accuracy of our classification method. According to Bell et al. who used a classification method similar to ours, the accuracy of the method was 94–96% for cases of breast cancers [16].

Our study was also limited by the fact that we did not measure the strength of backscattered signal but just calculated the area with Doppler signal within each HCC nodule. Therefore, our results did not reflect differences in the strength of Doppler signals. Estimation of flow velocity by comparing the color of a pixel in CD US with a look-up table with a reference color bar at the side of the

Table 1. Peak %PDA of 10 HCCs on conventional and harmonic PD US and their time to peak %PDA after contrast injection

Case	Conventional PD US		Harmonic PD US	
	Time (s)[a]	%PDA[b]	Time (s)[a]	%PDA[b]
1	30	16.6	40	23.3
2	50	33.5	60	46.7
3	60	49.0	20	47.5
4	60	42.6	60	28.4
5	60	76.4	90	46.2
6	30	61.7	40	35.0
7	90	57.8	40	27.9
8	50	20.8	90	6.9
9	40	51.2	50	11.0
10	90	6.6	60	0.8

[a] Time, time to peak %PDA after contrast injection
[b] %PDA, percentage of area with power Doppler signal versus total area of nodule of HCC

Table 2. Mean %PDA of ten nodules of HCC on conventional and harmonic PD US at each scan time

Time (s)[a]	Mean %PDA		Ratio (C/H)[b]	*p*
	Conventional PD US	Harmonic PD US		
0	6.9	0.4	17.3	0.018
20	15.0	11.1	1.4	0.3
30	25.8	12.9	2.0	0.0
40	30.4	16.6	1.8	0.0
50	34.1	16.5	2.1	0.0
60	34.9	19.5	1.8	0.0
90	28.5	15.6	1.8	0.0
120	24.6	12.2	2.0	0.0
150	21.6	7.6	2.8	0.0
180	19.9	5.5	3.6	0.0
240	13.3	2.0	6.7	0.0
300	8.6	1.2	7.2	0.0

[a] Time, time after contrast injection
[b] Ratio (C/H), ratio of mean %PDA on conventional PD US to mean %PDA on harmonic PD US

CD US image has been reported [16]. Similarly, the strength of PD signal may be quantified by comparing the color of a pixel in PD US with the reference color bar at the side of PD US image. In this study, we did not assess the strength of the Doppler signal because we thought that its strength was affected more by the color gain setting than by %PDA. Although our method has some limitations, we believe that it is superior to subjective methods of visually assessing the Doppler signal using crude descriptive criteria.

In conclusion, contrast-enhanced harmonic PD US may be an effective method in the evaluation of tumor vascularity of HCC because it produces fewer artifacts. However, in harmonic PD US, the area of PD signals within the tumor is smaller and the duration of effective enhancement is shorter than with conventional PD US. A good understanding of the difference between two contrast-enhanced PD US techniques may help in the proper evaluation of tumor vascularity of HCC when using PD US and a contrast agent.

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