The role of the quantitative method in the assessment of hepatocellular carcinoma wash-in and wash-out phases by contrast enhanced ultrasound

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Summary

Objective: To quantitative analysis the wash-in and wash-out of hepatocellular carcinoma (HCC) by contrast enhanced ultrasound and to find out the correlation between these characterizations with tumor size and tumor differentiations. *Subject and method:* This study included 134 patients with 134 HCC nodules confirmed by histopathology from February 2014 to May 2017. All patients were evaluated by contrast enhanced ultrasound with contrast agent SonoVue (Bracco Company, Netherlands), using US machine (Philips EPIQ 5G, US) that has contrast programe including contrast auto-tracking software ROI, following the Guideline of WFUMB and EFSUMB 2012. *Result:* There were undifferentiated between HCCs with level's size (< 20mm, 20 - < 40mm, ≥ 40mm), the well-differentiated HCCs and the moderately to poorly HCCs from time to peak (TP) and enhancement slope (ES). However, there were differentiated between HCCs with level's size (< 20mm, 20 – < 40mm, ≥ 40mm), the well-differentiated HCCs and the moderately to poorly HCCs from wash-out time and clearance slope with statistically significant (p<0.05). *Conclusion:* The HCCs nodules showed a "fast-in" in the artery phase and "fast-out" in the portal and late phase. The wash-out time and clearance slope correlate with HCCs's size and play an important role in predicting the differentiation of HCCs.

Keywords: Hepatocellular carcinoma, contrast enhanced sonography.

1. Background

Hepatocellular carcinoma is a hypervascular tumor, so ultrasound using contrast enhanced agent usually shows fast infiltration in the hepatic artery phase and fast eliminates in the portal and late phase. The image ultrasound of quickly increase in arterial phase and decrease in sound compared to liver parenchyma in portal phase and late phase. Previously, the method used to compare image of amplification or reduction of sound to assess the rapid infiltration or elimination properties of the tumor. Currently, the world has used quanlitative methods to evaluate through sound absorbing indices such as TP (time to peak), PI (peak intensity intensity), ES (enhancement slope) to assess the extent of fast infiltration. HCC inhibitors and rapid elimination indicators such as wash out time (WOT) and clearance slope (CS).

Currently with the development of the new generation of contrast agents as SonoVue (Bracco, Milan, Italy) is widely available in Europe and Asia (China, Korean…), replacing levovist for radiology application. So that contrast enhanced ultrasound (CEUS) is the quantitative diagnostic method that using ROI software having become common.

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The using of CEUS in diagnosis of hepatocellular carcinoma has been suggested in guidelines in the World Health Organization's Guidelines for the Diagnosis and Treatment of Hepatologists and Associations in the world, such as the Japan Society of Hepatology - 2014 (JSH), the Asia Pacific Association for the Study of Liver Disease - 2010 (APASL)...

The purpose of this study was to analyze the hepatocellular carcinoma (HCC) with quantitative analysis of the wash-in and wash-out of hepatocellular carcinoma (HCC) by contrast enhanced ultrasound and to find out the correlation between these characterizations with tumor size and tumor differentiations.

2. Subject and method

2.1. Subject

From February 2014 to May 2017, 134 patients were included in this study. All the patients were prospectively selected on the basic of ultrasound and histopathology examination. Written informed consent was obtained from all patients, and the study was approved by the Ethical Commitee of the 108 Military Central Hospital. The maximum diameters of the HCC nodules ranged from 14mm to 120mm (mean, 43.7 ± 21.0mm) and divided into 3 size's groups: Under 20mm (14 nodules), from 20mm to under 40mm (50 nodules) and from 40mm (70 nodules). The diagnoses for all 134 nodules were confirmed by histopathologic examination with the specimens obtained by ultrasonography guided biopsy (n = 96) or surgical resection (n = 38).

2.2. Method

Equipment: The ultrasonographic equipment used in this study was an EPIQ 5G machine (Philips, US) with a convex transducer with frequency of 3.5MHz. The contrast - specific software with the low mechanic index (MI = 0.06) in the system. The contrast agent used in this study was SonoVue (Bracco, Amsterdam, Netherlands), a sulfur hexafluoride-filled microbubble contrast agent. A volume of 2.4mL of SonoVue was injected into the antecubital vein in a bolus fashion (within 1 - 2 seconds) through a 20-gauge intravenous cannula, folowed by a flush of 10mL of 0.9% normal saline solution.

Ultrasonographic examination

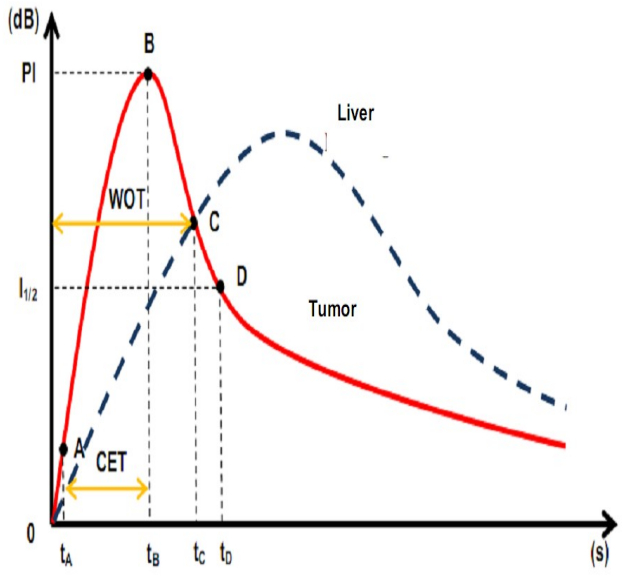
The scanning was performed by one investigator, who had experience in CEUS. Before administration of the contrast agent, baseline ultrasonography was performed to scan the liver thoroughly. All nodules were located less than 10cm from the body surface. When the target lesion was located, the imaging settings such as gain, depth, and focal zone were optimized. Generally, a single focal zone was placed at the bottom of the nodules to be assessed. The maximum diameter of each nodules was measured, and the liver segment location was determined according to the Couinaud [8] and Bismuth [2] classification systems. Digital cine clips were stored on the personal computer that connected to the US machine by way of a high performance, hardware-based and real-time. The CEUS's processing was divided into 3 phases following the "Guidelines and Good Clinical Practice Recommendations for Contrast Enhanced Ultrasound (CEUS) in the Liver - Update 2012", including arterial phase (start: 10 - 20 seconds, end: 30 - 45 seconds), portal phase (start: 30 - 45 seconds, end: 120 seconds) and late phase (> 120 seconds). The enhancement patterns in the 3 phases were subdivided into hypo-enhancement, iso-enhancement, and hyper-enhancement compared with the surrounding liver parenchyma.

Quantitative analysis in contrast-enhanced ultrasonography

The EPIQ 5G's quantification software was applied to derive the enhancement curves of different patients. This software is designed for real-time evaluation of tissue perfusion obtained by CEUS scanning. Regions of interest (ROI) were defined in the interior of HCCs corresponding to no less than 20% of the whole mass from the frame corresponding to the best identification of the lesion during arterial phase; doing so helped to avoid large vessels, necroses and the edge of HCC nodules. All considered signals were derived from echo-power values that were estimated following

log-compressed video images used for best-fit quantification, and echo-signal quantification was initiated as soon as microbubbles were visualised by eliminating the baseline frames, after which the signals were smoothed with a parametric model for bolus kinetics. A modified log-normal distribution was used for best-fit optimisation.

A series of parameters, including the time to peak (TP, the interval from time 0 to the image where the intensity in the region of interest is maximal, second -s), peak intensity (PI, the value of the maximum intensity in the region of interest that occurs after time 0, dB), contrast-enhanced time (CET, the difference between the time to peak and arrival time as *tc* in graphic, unit by second - s), wash-out time (WOT, the time from time 0 to the time when the intensity in the region of interest is lower than that in the surrounding parenchyma, as *tc* in graphic, unit by second - s), enhancement slope (ES, the ratio of peak intensity to the contrast-enhanced time, dB/s), and clearance slope (CS, the ratio of the difference between peak signal intensity and 50% signal intensity to the time interval from the peak to 50% signal intensity, dB/s), were used to evaluate the contrast enhancement patterns of the focal nodules.



Graphic 1. The diagram illustrating the parameters

Histopathologic evaluation: All histopathologic specimens were reviewed by a single pathologist who was blinded to the contrast-enhanced sonographic findings. The degree of cellular differentiation (well, moderate, or poor) was categorized according to the World Health Organization classification. Of these 134 HCCs, 48 nodules had well-differentiated (36.2%), 68 nodules had moderately differentiated (50.4%) and 18 nodules had poorly differentiated (13.4%). All the nodules were divided into 2 groups: Group 1 (well-differentiated hepatocellular carcinomas) and group 2 (moderately to poorly differentiated hepatocellular carcinomas).

Statistical analysis: A Student T-test were used to compare the differences in perfusion parameters between the groups. A $\chi 2$ test was used to compare the enhancement patterns between the groups. Quantitative data are expressed as mean ± SD. $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS version 20.0 for Windows software.

3. Result

Table 1. The characterization of patients

Number of patients	134	
Number of tumors	134	
Sex	Male	117 (87.3%)
	Female	17 (12.7%)
	Male/Female	~ 6.88/1
Age (years)	Mean	60.4 ± 12.5
	Max	82
	Min	22

Table 2. The characterization of pathology

Pathology's characteristics	Number (%)
Well-differentiated	48 (36.2)
Moderately differentiated	52 (50.4)
Poorly differentiated	18 (13.4)

Table 3. The group's size for hepatocellular carcinomas

Hepatocellular carcinoma	Number (%)
< 20mm	48 (42.1)
20 - < 40mm	52 (45.6)
≥ 40mm	14 (12.3)

Table 4. The parameters "fast in" and "fast out" CEUS by ROI software for of HCC

Parameters	$\bar{X}$ ± SD
AT (s)	9.26 ± 0.92
TP (s)	21.07 ± 2.97
PI (dB)	26.66 ± 5.73
WOT (s)	50.54 ± 21.75
ES (dB/s)	2.35 ± 0.64
CS (dB/s)	0.69 ± 0.22

Using of the quantitative methods in evaluating the floe intratumoral by the autotracking contrast quantification curve. There were not differentiation between groups with tumor size (< 20mm, 20 - < 40mm, ≥ 40mm) from the time to peak (TP) and enhancement slope (ES) in our study. However, there were differentiation between this groups from the wash-out time (WOT) and clearance slope (CS) with statistically significant ($p<0.05$) (Table 5), the similar result with the groups of differentiation. For HCCs difderentiated cells, we divided the HCCs enhanced pattern (134 nodules) into two groups of differentiation as well-differentiated (49 nodules) and moderately to poorly differentiated (84 nodules) (Table 6).

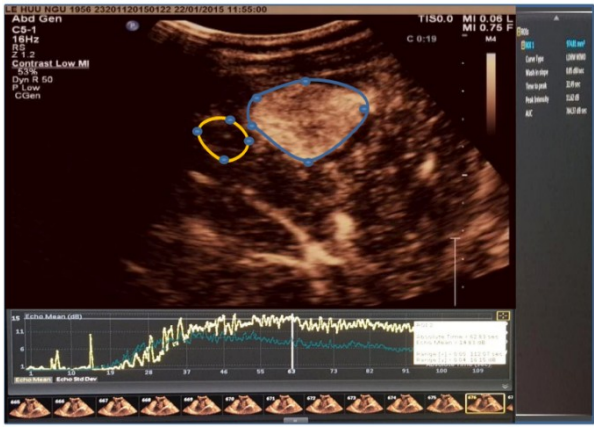


Figure 1. HCC showed fast-in and fast-out by the ROI quantitative methods

Table 5. The correlation parameters by "ROI" software and the size of HCC

Size / Parameters	< 20mm(1) (n = 14) ± SD	20 – <40mm(2) (n = 50) ± SD	≥ 40mm(3) (n = 70) ± SD	p (T- test)
AT (s)	8.97 ± 0.66	9.19 ± 0.88	9.37 ± 0.98	>0.05*
TP (s)	21.59 ± 2.82	21.32 ± 3.69	20.79 ± 2.39	>0.05*
PI (dB)	29.19 ± 4.94	25.81 ± 5.43	26.76 ± 5.98	>0.05
WOT (s)	57.33 ± 27.26	53.36 ± 22.33	47.16 ± 19.78	>0.05[a] <0.05[b] >0.05[c]
ES (dB/s)	2.48 ± 0.64	2.27 ± 0.71	2.39 ± 0.59	>0.05*
CS (dB/s)	0.62 ± 0.25	0.66 ± 0.19	0.73 ± 0.23	>0.05[a] <0.05[b] >0.05[c]

Table 6. The correlation parameters by "ROI" software and the differentiated of HCC

Differentiated / Parameters	Well (n = 48) ± SD	Moderately to poorly (n = 86) ± SD	p (T- test)
AT (s)	9.10 ± 0.77	9.36 ± 0.98	> 0.05
TP (s)	21.91 ± 3.10	20.60 ± 2.81	> 0.05

PI (dB)	28.45 ± 5.12	25.66 ± 5.82	< 0.05
WOT (s)	58.40 ± 24.12	45.46 ± 18.48	< 0.05
ES (dB/s)	2.36 ± 0.62	2.35 ± 0.66	> 0.05
CS (dB/s)	0.63 ± 0.22	0.73 ± 0.21	< 0.05

4. Discussion

Hepatocellular carcinomas in a hypervascular tumor, so the contrats agents will rapidly infiltrate in the arterial phase after intravenous injection. According to Blondi et al reported that almost all hepatocellular carcinoma showed intratumoral enhancement in the arterial phase, however some of them showed hypovascular with the size under 20mm showed non-enhancemend in the arterial phase and this rate decreased to zero percent with the tumor increased of size over 20mm [2]. On pathology, Choi et al had found that the tumor vascular involved in tumor size and developmental stage [3].

Hepatocellular carcinoma usually showed enhancement fast-in and fast out by the hyper-enhancement in the arterial phase and hypo-enhancement in the portal phase and in the late phase. This result with the time to peak (TP) as 21.07 ± 2.97s (Table 4) which was due to the hepatocellular carcinoma being supplied mainly by the arteries and the intratumoral abnomal arteries as: Morphology, vascular structure, flow's velocity, resistance, intratumoral arteriovenous shunt... [7]. So that hepatocellular carcinoma enhanced faster and earlier than parenchymal liver with the alerance slope as 0.69 ± 0.22dB/s, the wash-out time as 50.54 ± 21.75 seconds was due to portal phase (from 45 to 120 seconds) (Table 4).

The fast wash-out of the contrast agents in the hepatocellular carcinoma related to the tumor size and differentiation cells expressed by difference in hypo-enhancement in the portal phase. The tumor with large size have more malignant and its ratio of intratumoral arteriovenous shunt is higher than small tumor, this is one of the causes made the big hepatocellular carcinoma wash-out faster. According to Mohamed AM and Barron RL, the intratumoral arteriovenous shunt rate increases in proportion to the degree of malignancy [1], [9]. Besides, the role of Kupffer cell also affects the wash-out, following by Inoue et al showed that the tumors with iso-enhancement had more Kupffer cells than the tumors with hypo-enhancement in portal phase.

In addition to evaluating the wash-in and wash-out of hepatocellular carcinoma by the above quantitative method, we also use quantitative methods based on hemodynamics of the tumor through the variation of contrast agents over the time. The wash-out time of the HCCs with size ≥ 40mm by the wash-out time (WOT) as were 47.16 ± 19.78s and the clearance slope (CS) as were 0.73 ± 0.23dB/s, were faster than the HCCs with size < 20mm by the WOT as were 57.33 ± 27.26s and the CS as were 0.62 ± 0.25dB/s. As the similar, the wash-out time of the morderately to poorly differentiated HCCs by the WOT as were 45.46 ± 18.48s and the CS as were 0.73 ± 0.21dB/s, were faster than well-differentiated HCCs by the WOT as were 58.40 ± 24.12s, and the CS as were 0.63 ± 0.22dB/s) ($p<0.05$) (Table 5, Table 6). In the study of Xu et al showed that the use of quantitative methods shows the difference of hepatocellular carcinoma by size and differentiation of cells [10].

Hepatocellular carcinoma usually occurs with liver cirrhosis. Carcinogenesis is often a multistep procedure in cirrhosis. This procedure includes progression from a cirrhotic nodule, to a macroregenerative nodule, to a high-grade dysplastic nodule, to a low-grade dysplastic nodule, to frank hepatocellular carcinoma [6]. Progression along the multistep pathway is characterized by cytologic and architectural changes in the vascular due to differentiated cell [4], [11].

The grade of tumor cell differentiation is an important prognostic factor for hepatocellular carcinoma, and histopathologic examination is the only way to clarify the differentiation. With the development of second generation sonographic

contrast agents and contrast specific imaging techniques, real-time contrast-enhanced sonography can be used to visualize lesion perfusion. Contrast enhanced sonography has become a useful and less invasive method for characterizing and evaluating the intranodular blood supply of focal liver lesions, and it can indicate pathologic features of hepatocellular carcinomas preoperatively [10].

5. Conclusion

By the quantitative method showed the HCCs nodules "fast-in" in the arterial phase and "fast-out" in the portal and late phase. The wash-out time (WOT) and clearance slope (CS) correlate with HCCs's size and play an important role in predicting the differentiation of HCCs.

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