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Quantitative analysis of contrast-enhanced ultrasonography following living donor liver transplantation: early diagnosis of middle hepatic venous occlusion

Jiun Im[1], Woo Kyoung Jeong[1,2], Min Woo Lee[1,2], Young Kon Kim[2], Ji Hye Min[2], Jong Man Kim[3], Gyu Seong Choi[3], Jae Won Joh[3]

[1]Department of Medical Device Management and Research, Samsung Advanced Institute for Health Sciences & Technology (SAIHST), Graduate School of Sungkyunkwan University, [2]Department of Radiology and Center for Imaging Sciences, and [3]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Abstract

Aim: This study aimed to evaluate whether a quantitative contrast-enhanced ultrasonography (CEUS) study is feasible to diagnose middle hepatic venous occlusion after living donor liver transplantation (LDLT). **Materials and methods**: From December 2018 to July 2019, the CEUS study on the first postoperative day had been conducted in patients who underwent LDLT. 46 patients were finally included in the study. To obtain CEUS parameters from time-intensity curves (TICs) on the hepatic parenchyma, the two regions of interests (ROIs) were located in the right hepatic vein (RHV) territory and middle hepatic vein (MHV) territory of the right hepatic graft. The measured CEUS parameters were wash-in slope (WIS), peak intensity (PI), time to peak (TTP), and area under the curve (AUC). The subjects were classified into the occlusion and non-occlusion groups. In each group, the parameters measured in the RHV and MHV territories were compared with paired-sample Student's *t*-tests. **Results**: Hepatic venous occlusion was diagnosed in 25 patients (54%). The WIS, TTP, and AUC of the MHV territory (2.95 dB/sec; 22.39 sec; 204.27 dB·sec, respectively) were significantly different from those of the RHV territory (2.16 dB/sec; 25.81 sec; 165.66 dB·sec; all $p<0.05$). There were no statistically significant differences in PI between the MHV and RHV territories (19.08 dB vs. 18.27 dB, respectively; $p=0.259$). In the non-occlusion group, there was no parameter which was significantly different between MHV and RHV territories ($p>0.05$). **Conclusion**: The parametric analysis of CEUS can help diagnose middle hepatic venous occlusion after LDLT.

Keywords: Liver transplantation; hepatic veins; venous thrombosis; ultrasonography; microbubbles

Introduction

Liver transplantation (LT) is widely accepted as a curative treatment method for hepatocellular carcinoma (HCC) or end-stage liver diseases, resulting from underlying diseases such as cirrhosis, viral hepatitis, alcoholic liver disease, and fulminant hepatitis [1-4]. In recent years, the number of living donor liver transplantation (LDLT) has been increasing regardless of East and West. At first, it was preferred in Asian countries due to cultural and social differences; but now, due to the increase in the number of waiting for LT, organ shortage have worsened and the interest in LDLT has increased [1,5].

As the surgical techniques for LDLT have improved, donors and recipients have become safer after the surgery. For example, a recipient who has received a right liver graft is vulnerable to hepatic congestion if the middle hepatic vein (MHV) is not reconstructed. However, the MHV is usually left with the donor because the do-

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Corresponding author: Woo Kyoung Jeong, MD, PhD
Department of Radiology and Center for Imaging Science, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Phone: 82-2-3410-1923
Fax: 82-2-3410-0049
E-mail: jeongwk@gmail.com

nors' safety is a top priority. Thus, venous branches (V5 and V8) of anterior segment of right liver graft are connected to the recipients' vena cava, modified right liver graft technique. It was designed for providing similar functioning liver mass as the draining of anterior section is preserved by connecting the V5 and V8 to vena cava through interposition venous graft generally made by cryopreserved deceased donor iliac vein [6].

Due to the complexity of the surgical technique of LDLT, as written above, venous thrombosis at the interposition vein graft frequently occurs as a complication after the right liver LDLT and the venous complication may lead to the graft loss [7]. To diagnose this vascular complication early, contrast-enhanced ultrasound (CEUS) and Doppler ultrasound (US) are performed during the immediate postoperative period to detect vascular complication [2,8-10].

In hepatic venous stenosis, CEUS shows a high echogenicity of the involved segment in the arterial phase and low echogenicity in the portal phase. The accuracy of CEUS is relatively higher than that of Doppler US [11]. However, the features are translated subjectively and can be affected by the user's examination technique. Hence, quantitative results of CEUS that can supplement these results are needed.

Our study hypothesizes that the time-intensity curve (TIC) analysis using CEUS can reveal the difference of the perfusion status of MHV territory compared with that of the right hepatic vein (RHV) territory quantitatively in patients with interposition vein graft occlusion. This study aimed to find significant quantitative parameters that indicate interposition graft vein occlusion with the TIC of CEUS and to compare the group without venous thrombosis.

Materials and method

Data collection

The institutional review board of our institution approved this retrospective study (IRB No. 2019-10-135) and the informed consent requirement could be waived.

From December 2018 to July 2019, the LDLT patient database was reviewed to find eligible patients. Inclusion criteria were as follows: 1) the patients underwent CEUS study on the first postoperative day (POD); 2) video clips of CEUS image stack that recorded as a DICOM format were available. Among them, we excluded the patients who met the following exclusion criteria: 1) the patients who underwent left liver LDLT without middle hepatic vein reconstruction; 2) paediatric LDLT; 3) poor CEUS image quality to analyse due to motion artifact (mainly due to respiration) (fig 1).

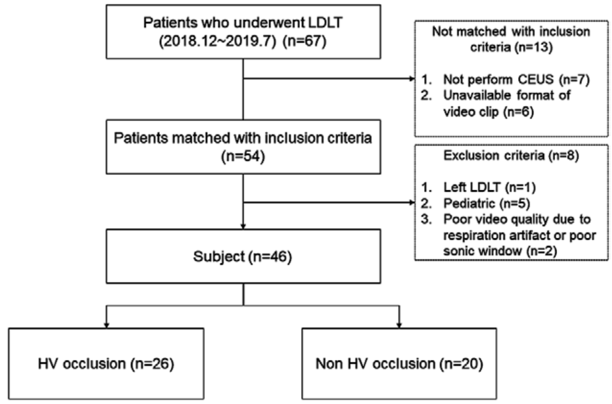

Fig 1. Flowchart of the subject enrolment

US examination including Doppler and CEUS

On the first POD, protocol CEUS and Doppler study were performed by a board-certified radiologist with 16-year experience, to detect the acute vascular complication. The study data were realised using Philips EPIQ7 ultrasound equipment with C2-9 transducer (Philips Healthcare, Netherland). The Doppler US was followed by the CEUS study: First, grayscale US was performed to explore the hepatic parenchymal change, the presence of thrombus in the grafted vein and the presence of hematoma around the hepatic graft. Second, colour Doppler US was performed to investigate the presence of vascularity in the vascular structures. Subsequently, pulsed Doppler US was performed to measure Doppler parameters of hepatic artery and portal vein such as hepatic artery resistive index, hepatic artery systolic acceleration time and portal venous flow velocity at the pre- and post-anastomotic sites. The examiner also observed hepatic venous waveform at anastomosis of the RHV, right inferior hepatic vein (RIHV), MHV tributaries (V5 and V8) and interposition venous graft. During the surgery, the MHV was reconstructed with cryopreserved veins as interposition venous grafts. In general, if the MHV tributary diameter was higher than 5 mm, it was anastomosed to the interposition venous grafts; small branches (≤5 mm in diameter) were ligated.

After the Doppler study, the same examiner subsequently performed the CEUS study using the second-generation contrast agent (Sonovue; sulfur hexafluoride; Bracco Diagnostics; Milan, Italy) diluted with saline - 2.4 mL of the contrast agent solution was injected into the left antecubital vein. Real-time CEUS with a low mechanical index (0.079) was started and video clips were recorded simultaneously for 30 seconds after the microbubble injection started. The hepatic graft and blood vessels were observed through intercostal spaces during arterial and portal venous phases.

Imaging analysis

Imaging data were extracted from the video clips in DICOM format to Q-Lab software (Q-Lab; Philips Healthcare; Netherlands). One of the authors drew regions of interest (ROIs) at each RHV territory and MHV territory of the transplanted liver, avoiding major intrahepatic vessels. Three ROIs were located at the territory with a similar depth on the transducer surface to reduce the effect of ultrasound beam attenuation (fig 2). At each ROI, we obtained a TIC in the arterial phase for 25 seconds and measured parameters at the TIC. Before measuring the parameter, curve fitting was performed with a log-normal wash-in-wash-out method. The outliers of measurement at each time point were excluded from the dataset to reduce the patient's respiratory movement variation. The obtained CEUS parameters were wash-in slope (WIS), peak intensity (PI), time to peak (TTP), and area under the curve (AUC) of the TIC. The definition of each parameter was as following: WIS (unit: dB/sec) is the maximum rate of increase in intensity per time, during the wash-in period (before arrival to the PI) of the TIC, which means the speed of enhancement; PI (unit: dB) is the highest level of intensity on the TIC, which means a maximum number of microbubbles passing through the hepatic sinusoids per time. It represents the full blood flow in the liver; TTP (unit: sec) is the time taken for PI; and AUC (unit: dB·sec) is the total area under the TIC for 25 second, which represents blood volume in the hepatic parenchyma [12]. The mean values of parameters in three ROIs were calculated for statistical analysis.

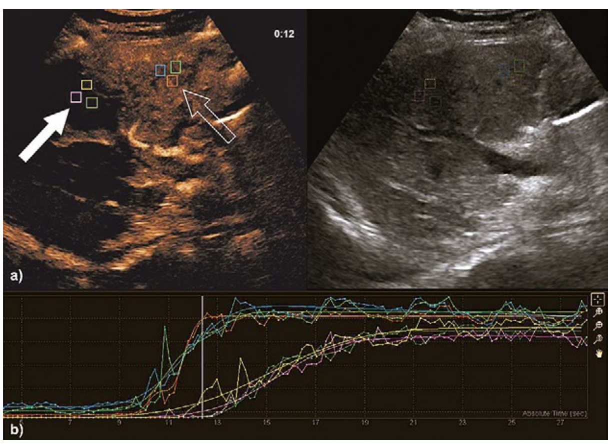

Fig 2. A 65-year-old woman with hepatitis B-related liver cirrhosis. During the operation, the tributaries of the middle hepatic vein were ligated without anastomosis (occlusion group). Three regions of interest (ROIs) are located in each right hepatic vein (RHV) territory (solid arrow) and middle hepatic vein (MHV) territory (open arrow) (a). The time-intensity curve (TIC) shows early wash-in and abruptly elevated enhancement in the ROIs of MHV territory, compared to those of RHV territory (b).

Occlusion versus non-occlusion groups

The subjects were classified into the hepatic vein occlusion group and non-occlusion group. The occlusion group was defined as the patients whom the branches of the middle hepatic vein were not connected to the surgery or occluded by thrombosis in the interposition graft vein, which was proved by Doppler ultrasound and/or postoperative CT study at the same time. The non-occlusion group was defined as the patients whom the middle hepatic vein tributaries were connected, and blood flow maintained at the Doppler and CEUS examination on the first POD.

Statistical analysis

Descriptive statistics are presented as the mean scores and standard deviations for continuous variables, and as frequency (percentage) for categorical variables. Clinical and imaging characteristics were compared between the occlusion and non-occlusion groups using the Wilcoxon rank-sum test and the chi-square test or Fisher`s exact test for categorical variables. CEUS parameters were divided by the location of ROIs, i.e. RHV territory or MHV territory. It compared in all subjects, the occlusion group, and the non-occlusion group. In addition, the paired-sample *t*-test was used to compare CEUS parameters between the RHV and MHV territories.

All statistical analyses were performed using MedCalc software (version 19.1.3; MedCalc Software bvba, Belgium). A p-value of less than 0.05 was considered indicative of a significant difference.

Results

A total of 67 patients who underwent LDLT during the study period and 54 patients who met the inclusion criteria were eligible for this study. Among the 54 patients, eight patients were excluded from the study, and a total of 46 patients (M:F=35:11) were finally included in the study. Among all subjects, 25 patients had proven occlusion of MHV tributaries and were allocated to the occlusion group. The other patients without hepatic vein occlusion (n=21) were allocated to the non-occlusion group.

The underlying diseases of patients in the occlusion group (18 men and 7 women; mean age, 56.6±6.8 years) were variable; the most common disease was HCC associated with hepatitis B viral infection (n=13; 52%) The other 21 patients belonged to the non-occlusion group (17 men and 4 women; mean age, 56.8±5.7 years) and the underlying disease of 15 patients (71%) was HCC associated with hepatitis B viral infection (n=15). Both on the first POD and the seventh POD, results of the liver function test were not different between two groups. The patients' detailed characteristics are summarized in Table I.

On Doppler US, most RHVs showed either triphasic or biphasic venous flow (n=46). The RIHV anastomosis was not occluded in the subsequent imaging studies in all patients (n=7) who received the RIHV anastomosis. In terms of MHV tributaries, all tributaries of MHV in the occlusion group showed no venous flow or monophasic flow in V5. All patients in the non-occlusion group showed either triphasic or biphasic flow in at least one of MHV tributaries (Table II).

Table III shows the mean comparison of CEUS parameters in all subjects. The PI in the RHV territory was significantly higher than that in the MHV territory (20.9 dB vs. 19.8 dB; p=0.038). The AUC of the RHV territory was slightly higher than that in the MHV territory; there was no statistical significance. The TTP and WIS were not different between the two territories.

In the occlusion group, the WIS of the MHV territory (2.95 dB/sec) was significantly higher than that of the RHV territory (2.16 dB/sec; p=0.008). The TTP of the MHV territory (22.39 sec) was shorter than that of the RHV territory (25.81 sec; p=0.017) and the AUC of the MHV territory (204.27 dB·sec) was significantly higher than that of the RHV territory (165.66 dB·sec; p=0.014). In contrast, the PI value in the MHV and RHV territories was not significantly different (19.08 dB vs. 18.27 dB; p=0.259). In the non-occlusion group, the differences be-

Table I. Demographic and laboratory data of the subjects

			Occlusion (n=25)	Non-occlusion (n=21)	p value
Age (years)			56.6 ± 6.8	56.8 ± 5.7	0.914
Male			18 (72)	17 (81)	0.483
Aetiology		HBV related HCC	13 (52)	15 (71)	0.605
		HCV related HCC	1 (4)	0	
		Alcoholic LC related HCC	2 (8)	2 (10)	
		HBV related LC	4 (16)	1 (5)	
		Alcoholic LC	2 (8)	2 (10)	
		Others†	3 (12)	1 (5)	
Laboratory data	POD 1	AST	559.4 ± 885.5	630.6 ± 1867.3	0.866
		ALT	466.2 ± 502.3	505.3 ± 1049.2	0.772
		Serum bilirubin	2.12 ± 1.82	1.83 ± 1.42	0.555
		Serum creatinine	0.90 ± 0.37	1.14 ± 0.91	0.222
	POD 7	AST	90.5 ± 116.9	91.8 ± 85.0	0.967
		ALT	241.2 ± 190.7	242.3 ± 182.7	0.985
		Serum bilirubin	1.58 ± 1.06	1.51 ± 1.20	0.824
		Serum creatinine	1.13 ± 0.81	1.26 ± 1.12	0.637

The results are expressed as number (%) or * means ± standard deviations. HV, hepatic vein; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LC, liver cirrhosis; LT, liver transplantation; POD, postoperative day; AST, aspartate transaminase; ALT, alanine transaminase. †Others: toxic hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis

Table II. Hepatic venous Doppler ultrasound findings of the subjects

	Occlusion group (n=25)			Non-occlusion group (n=21)		
	Tri/biphasic flow	Monophasic flow	No flow	Tri/biphasic flow	Monophasic flow	No flow
RHV	24	1	0	21	0	0
RIHV	3	0	0	3	1	0
V5	0	1	24	21	0	0
V8	0	0	25	10	0	0

The results represent number of patients. RHV, right hepatic vein; RIHV, right inferior hepatic vein

Table III. CEUS parameters measured in the RHV and MHV territories of the subjects

Parameters	MHV territory	RHV territory	Differences	p value
WIS (dB/sec)	2.68 ± 1.86	2.54 ± 3.37	-0.14 ± 2.55	0.718
PI (dB)	20.86 ± 13.22	19.83 ± 14.42	-1.03 ± 3.26	0.038
TTP (sec)	23.07 ± 5.73	24.82 ± 4.81	1.75 ± 6.50	0.075
AUC (dB·sec)	231.30 ± 112.38	211.23 ± 150.90	-20.07 ± 94.03	0.155

The results are expressed as means ± standard deviations. RHV, right hepatic vein; MHV, middle hepatic vein; AUC, area under the curve; PI, peak intensity; TTP, time to peak; WIS, wash-in rate tween all CEUS parameters in the two territories were not statistically significant (Table IV, fig 3).

Discussion

In patients with hepatic venous occlusion Doppler US shows no detectable flow in the thrombosed segment, weak and monotonous pattern of venous flow or reversed portal blood flow in the involved segment [13]. Previous studies have shown that sensitivity of Doppler US for venous obstruction diagnosis is 90-97% of and specificity 67-77% [14,15]. In other words, the high sensitivity of Doppler US is advantageous as a primary surveillance method for early detection of complications although the relatively high-false positive rate is its limitation.

The advantages of CEUS as a complementary test for detecting vascular complications are as follows. First, it is easy to examine hepatic vascular patency. In a hepatic artery with a small diameter, the examiner should take a long time to find the hepatic arterial flow using Doppler US only [16]. Second, the CEUS can show the parenchymal perfusion l such as other contrast-enhanced CT or MRI. In contrast to the grayscale US, it can also show the ischemic or congestive change of the hepatic parenchyma [14,17], CEUS can show the parenchymal difference by congestion more clearly. Early ischemic change of hepatic parenchyma can be seen in the delay phase of the CEUS study. In patients with hepatic congestion, hyperechoic in the involved tissue is more commonly seen in the grayscale US [14]. According to a previous study [11], which investigated the ability of CEUS to diagnose obstruction of MHV after right liver LDLT, the specificity of CEUS was higher comparing to Doppler US (97% vs. 86%; p=0.024) and the sensitivity was similar (91% vs. 83%). The qualitative analysis of CEUS showed good accuracy for diagnosing hepatic venous obstruction, but it still has a limitation regarding interobserver variability related to the operator's skill. Quantitative determination of hepatic perfusion on CEUS can overcome the existing limitations.

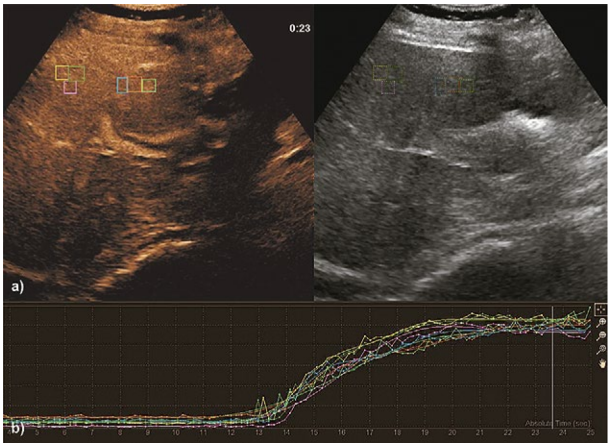

Fig 3. A 63-year-old woman with toxic hepatitis. The time-intensity curve (TIC) of patients without hepatic vein occlusion shows similar enhancement patterns between the parameters on the middle hepatic vein territory and right hepatic vein territory (a and b).

The results of the present study were explained following clinical hypotheses. Because TTP and WIS of the MHV territory were significantly shorter and higher than those of the RHV territory in patients with occlusion, it could explain the decrease in portal venous inflow and compensatory arterial hyperperfusion following venous obstruction [11,18,19]. In other words, the segment with hepatic congestion by an acute venous obstruction, such as MHV territory, would be enhanced earlier than the normal hepatic tissue, such as RHV territory. The increased AUC represented hepatic blood volume during the arterial phase of the MHV territory, which was also larger than that of the RHV territory.

The PI of the RHV territory was higher than that of the MHV territory. The reason was not apparent; however, it would be possible that the US visualisation of the RHV territory might be better than that of the MHV

Table IV. The difference of CEUS parameters measured in the occlusion and non-occlusion groups

Parameters		MHV territory	RHV territory	Differences	p value
Occlusion group	WIS (dB/sec)	2.95 ± 1.99	2.16 ± 1.21	-0.79 ± 1.36	0.008
	PI (dB)	19.08 ± 7.04	18.27 ± 7.37	-0.80 ± 3.47	0.259
	TTP (sec)	22.39 ± 6.16	25.81 ± 4.60	3.42 ± 6.69	0.017
	AUC (dB·sec)	204.27 ± 96.70	165.66 ± 69.86	-38.62 ± 73.11	0.014
Non-occlusion group	WIS (dB/sec)	2.36 ± 1.69	3.00 ± 4.83	0.64 ± 3.36	0.396
	PI (dB)	22.98 ± 18.03	21.68 ± 19.90	-1.30 ± 3.06	0.066
	TTP (sec)	23.88 ± 5.21	23.64 ± 4.90	-0.24 ± 5.82	0.855
	AUC (dB·sec)	263.47 ± 123.27	265.48 ± 199.22	2.01 ± 111.97	0.935

The results are expressed as means ± standard deviations RHV, right hepatic vein; MHV, middle hepatic vein; AUC, area under the curve; PI, peak intensity; TTP, time to peak; WIS, wash-in rate territory or because the right hepatic vein occlusion is extremely rare following the right liver LDLT [20], the intrahepatic circulation in the RHV territory would be better comparing to the other area of the transplanted liver. On Doppler US of the RHV, triphasic or biphasic venous flow was observed in most patients in this study.

However, there are several limitations to this study. First, the study design was retrospective and the sample size was relatively small. Second, as an issue of beam penetration, the ROI could only be obtained on the near part from the transducer surface (e.g., near the liver capsule). Third, the sampled data in the ROIs oscillated because of a respiratory excursion. Thus, CEUS parameters could be affected by respiratory motion. However, we tried to reduce the variation by respiration by curve fitting and exclude the outlier of measurement at each time point. The ROI with automatic tracking of respiratory motion might be helpful in the subsequent study. Fourth, in this study, only the arterial phase was analysed. Patient respiration and contrast agent destruction at portal and delay phases were difficult to acquire for two minutes. Last, because a mechanical injector was not used, there may be some differences in the distribution of contrast medium between patients, and TIC measurements may also have a very small effect.

In **conclusion,** CEUS with quantitative analysis may help identify hepatic venous occlusion in patients who have undergone right liver LDLT. Moreover, TIC parameters, including TTP, WIS and AUC, can objectively help in diagnosing the hepatic venous occlusion.

Conflict of interest: none

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