

Contrast-Enhanced Ultrasonography in Evaluation of the Therapeutic Effect of Chemotherapy for Patients with Liver Metastases

Naoyuki Ueda,* Haruki Nagira,* Naoko Sannomiya,* Saeko Ikunishi,* Yuiko Hattori,* Akira Kamida,* Yuki Koyanagi,* Kenta Shimabayashi,* Kengo Sato,* Hiroaki Saito† and Yasuaki Hirooka*

*Department of Pathobiological Science and Technology, School of Health Science, Tottori University Faculty of Medicine, Yonago 683-8503, Japan and †Division of Surgical Oncology, Department of Surgery, School of Medicine, Tottori University Faculty of Medicine, Yonago 683-8503, Japan

ABSTRACT

Background The therapeutic effect of chemotherapy for liver metastases is currently determined by changes in tumor diameter depicted on computed tomography (CT) and magnetic resonance imaging, but it cannot accurately determine if there is central necrosis. Furthermore, due to the risk of radiation exposure and high cost, frequent examination using these methods places a heavy burden on patients. Meanwhile, real-time observation of blood flow and vessel morphology within tumors has become possible by contrast-enhanced ultrasonography (CEUS). However, use of CEUS in evaluating the therapeutic effect of anticancer chemotherapy has rarely been investigated. This study investigated whether changes in the time-intensity curve (TIC) of CEUS are useful indicators of the therapeutic effect of chemotherapy.

Methods Five patients with liver metastases who had undergone CEUS before and after chemotherapy were included in this study. The TIC of each time point was prepared to examine whether the following five TIC parameters serve as indicators of the therapeutic effect of chemotherapy: peak intensity, time to wash-in, time to peak intensity, slope of wash-in, and area under the curve. In each parameter, rate of change (ROC) was calculated by the expression [(values before chemotherapy minus those after chemotherapy)/those before chemotherapy × 100(%)].

Results (i) Among the five TIC parameters tested, ROC of the slope of wash-in and the area under the curve reflected the therapeutic effect of chemotherapy better than the remaining three parameters. (ii) TIC parameters after one cycle of chemotherapy were examined in two of five patients, and changes in the slope of wash-in and the area under the curve were in good agreement with the computed tomography findings indicative of the

therapeutic effect after the fourth chemotherapy cycle.

Conclusion The findings of this study suggest that ROC of the slope of wash-in and the area under the curve of the TIC are useful in evaluating the therapeutic effect of chemotherapy. Furthermore, there is a possibility that TIC analysis may enable early prediction of the therapeutic effect.

Key words liver; neoplasm metastases; Sonazoid; time intensity curve; ultrasonography

The therapeutic effect of chemotherapy for liver metastases is currently determined by changes in tumor diameter depicted on computed tomography (CT) and magnetic resonance imaging, but it cannot accurately determine if there is central necrosis. Frampas et al. reported that it was possible to predict the therapeutic effect by the change in tumor blood flow using dynamic CT.¹ However, due to the risk of radiation exposure and high cost, frequent examination using these methods places a heavy burden on patients.

Meanwhile, real-time observation of blood flow and vessel morphology within tumors has become possible by contrast-enhanced ultrasonography (CEUS). Lassau et al.^{2,3} reported that the time-intensity curve (TIC) parameters obtained from CEUS of tumors correlated well with the prognosis. Furthermore, Frampas et al.¹ reported that the area under the curve (AUC), one of the TIC parameters, was useful for assessing the blood flow. However, use of CEUS in evaluating the therapeutic effect of anticancer chemotherapy has rarely been investigated. This study investigated whether changes in the TIC of CEUS are useful indicators of the therapeutic effect of chemotherapy. Lassau et al. reported that the TIC parameters obtained from CEUS of tumors correlated well with the prognosis.^{2,3} Furthermore, Frampas et al. reported that AUC, one of the TIC parameters, was useful for assessing the blood flow.¹ However, use of CEUS in evaluating the therapeutic effect of anticancer chemotherapy has rarely been investigated. This study investigated whether changes in the TIC of CEUS are useful indicators of the therapeutic effect of chemotherapy.

Corresponding author: Yasuaki Hirooka, MD, PhD
yhirooka@med.tottori-u.ac.jp

Received 2016 July 27

Accepted 2016 September 1

Abbreviations: AUC, area under the curve; CEUS, contrast-enhanced ultrasonography; CT, computed tomography; PD, progressive disease; PR, partial response; RECIST, response evaluation criteria in solid tumors; ROC, rate of change; TIC, time-intensity curve

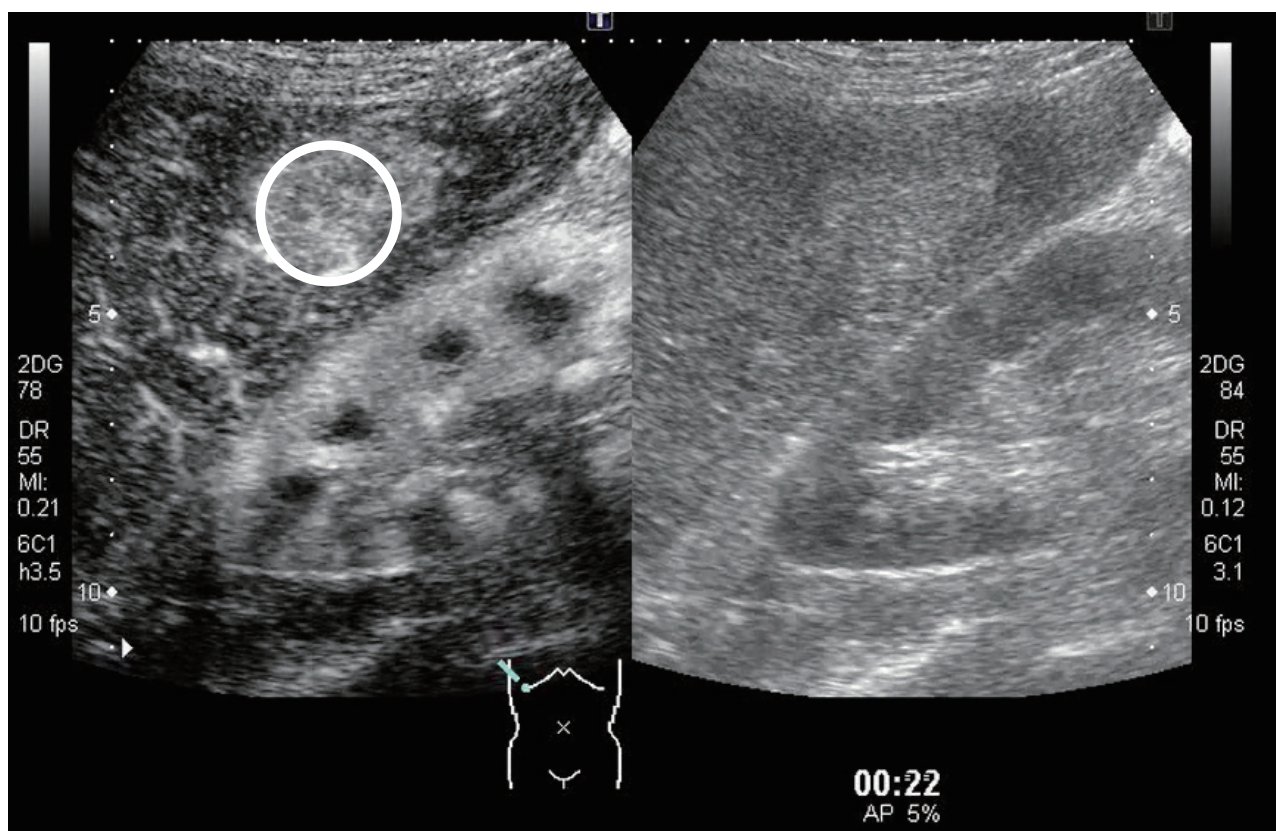


Fig. 1. ROI of the tumor. Circle indicates the ROI. Right image: B-mode. Left image: CEUS-mode. CEUS, contrast-enhanced ultrasonography; ROI, region of interest.

SUBJECTS AND METHODS

Five patients with liver metastases from digestive tract cancer underwent CEUS before and after chemotherapy from February 2011 to February 2015 in Tottori University Hospital and were followed up after chemotherapy. The site of the primary tumor was the rectum in two patients, stomach in two, and esophagus in one. Histological types of all primary tumor were adenocarcinoma.

Ultrasonography was performed using a Aplio XG (Toshiba, Tokyo, Japan) and a 3.5 MHz convex probe (PVT-375BT). Before CEUS, B-mode ultrasonography of the whole tumor was performed to determine the slice of images. Then the largest diameter slice of the tumor image was selected. A probe was fixed to obtain an image similar to the preselected image, and imaging was recorded for 3 min immediately after bolus injection of Sonazoid (Daiichi Sankyo, Tokyo, Japan) into the medial cubital vein. Ultrasound conditions (gain, dynamic range, mechanical index, depth, and focus) were unaltered throughout imaging of a particular patient. CEUS was performed before and after chemotherapy; the number of chemotherapy cycles ranged from one to five depending on the patient's condition.

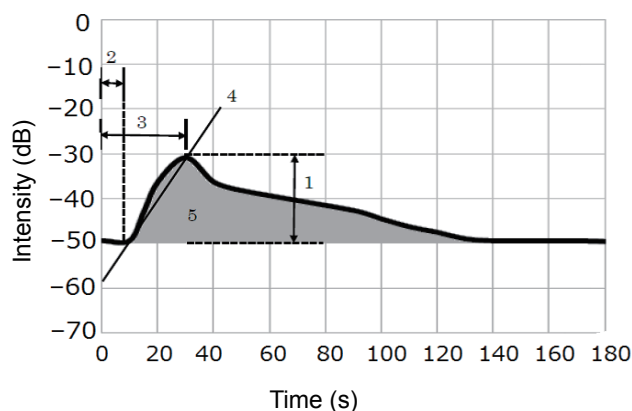


Fig. 2. TIC parameters. 1) Peak intensity. 2) Time to wash-in. 3) Time to peak intensity. 4) Slope of wash-in. 5) Area under the curve. TIC, time-intensity curve. dB, decibel.

ImageLab software ver. 2.9 (Toshiba, Tokyo, Japan) was used for image analysis. The region of interest was set to surround the entire region of the tumor (Fig. 1).

A TIC was prepared for each patient for 3 min immediately after Sonazoid injection. Changes in TIC param-

ters between before and after chemotherapy were examined in relation to the CT changes in tumor diameter.

The following TIC parameters were examined: peak intensity, time to wash-in, time to peak intensity, slope of wash-in (slope from baseline to peak), and area under the curve (Fig. 2). In each parameter, rate of change (ROC) was calculated by the expression, [(values before chemotherapy minus those after chemotherapy)/those before chemotherapy × 100(%)].

The therapeutic effect on the tumors was determined based on the CT changes in tumor diameter in accordance with the response evaluation criteria in solid tumors (RECIST) complete loss of the target lesion (complete response), > 30% decrease in the sum of the diameters of the target lesion relative to the baseline value (partial response), > 20% increase and 5-mm increase in the sum of the diameters of the target lesion relative to the baseline value (progressive disease), and small changes that do not meet the above criteria (stable disease).⁴

This study was approved by the Ethics Committee of the Tottori University Faculty of Medicine (1508A020).

RESULTS

The therapeutic effect according to the RECIST was a partial response in two patients and progressive disease in three (Table 1).

Figures 3 and 4 show the CEUS images and TIC before and after chemotherapy in a patient whose CT images indicated a good therapeutic effect (Patient 1, a 67-year-old woman with primary rectal cancer).

CEUS was performed after one cycle and CT was performed after four cycles of XELOX therapy (capecitabine and oxaliplatin). CEUS images showed weak contrast enhancement inside the tumor in the arterial phase before chemotherapy and reduced contrast enhancement inside the tumor in the arterial phase after chemotherapy. CT images showed a reduction in tumor size from 57 to 38 mm (Fig. 3). ROC of the peak intensity, the slope of wash-in and the area under the curve were decreased, while that of the time to wash-in and time to peak intensity were increased (Fig. 4).

Figures 5 and 6 show the CEUS images and TIC before and after chemotherapy in a patient whose CT images did not show a therapeutic effect (Patient 3, a 76-year-old man with primary stomach cancer).

CEUS was performed after one cycle and CT was

Table 1. Patients' characteristics

Case No.		1	2	3	4	5	
Age		67 y	70 y	76 y	80 y	76 y	
Sex		Female	Female	Male	Male	Male	
Primary tumor (Histological type)		Rectum (Adenoca.)	Rectum (Adenoca.)	Stomach (Adenoca.)	Esophagus (Adenoca.)	Stomach (Adenoca.)	
Evaluation of the CT	Time point	4 cycles	3 cycles	4 cycles	7 cycles	5 cycles	
	Change in tumor diameter	57 mm →38 mm	54 mm →23 mm	27 mm →43mm	26 mm →39 mm	35 mm →56 mm	
	Therapeutic effect	PR	PR	PD	PD	PD	
Evaluation of the CEUS	Time point	1 cycle	3 cycles	1 cycle	3 cycles	5 cycles	
	Peak intensity	before	-37.25	-23.16	-35.24	-40.27	-27.24
		after	-53.24	-24.40	-36.79	-17.55	-18.45
		ROC	-43.9	-5.3	-4.4	56.4	32.2
	Time to wash in	before	19	9	17	32	19
		after	21	9	18	19	25
		ROC	5.3	0.0	5.8	-40.6	31.6
	Time to peak intensity	before	23	19	38	46	29
		after	30	14	22	30	44
		ROC	30.4	-26.3	-42.1	-34.8	51.7
	Slope of wash-in	before	5.69	4.14	1.18	0.81	1.58
		after	0.75	2.21	5.80	2.14	2.18
		ROC	-86.8	-46.6	391.5	164.2	38.0
	Area under the curve	before	19.30	17.51	5.65	14.42	22.77
		after	1.73	10.03	12.09	23.29	29.31
ROC		-91.0	-42.7	114.0	61.5	28.7	

Adenoca., adenocarcinoma; after, after chemotherapy; before, before chemotherapy; CEUS, contrast-enhanced ultrasonography; CT, computed tomography; No., number; PD, progressive disease; PR, partial response; ROC, rate of change; y, years old.

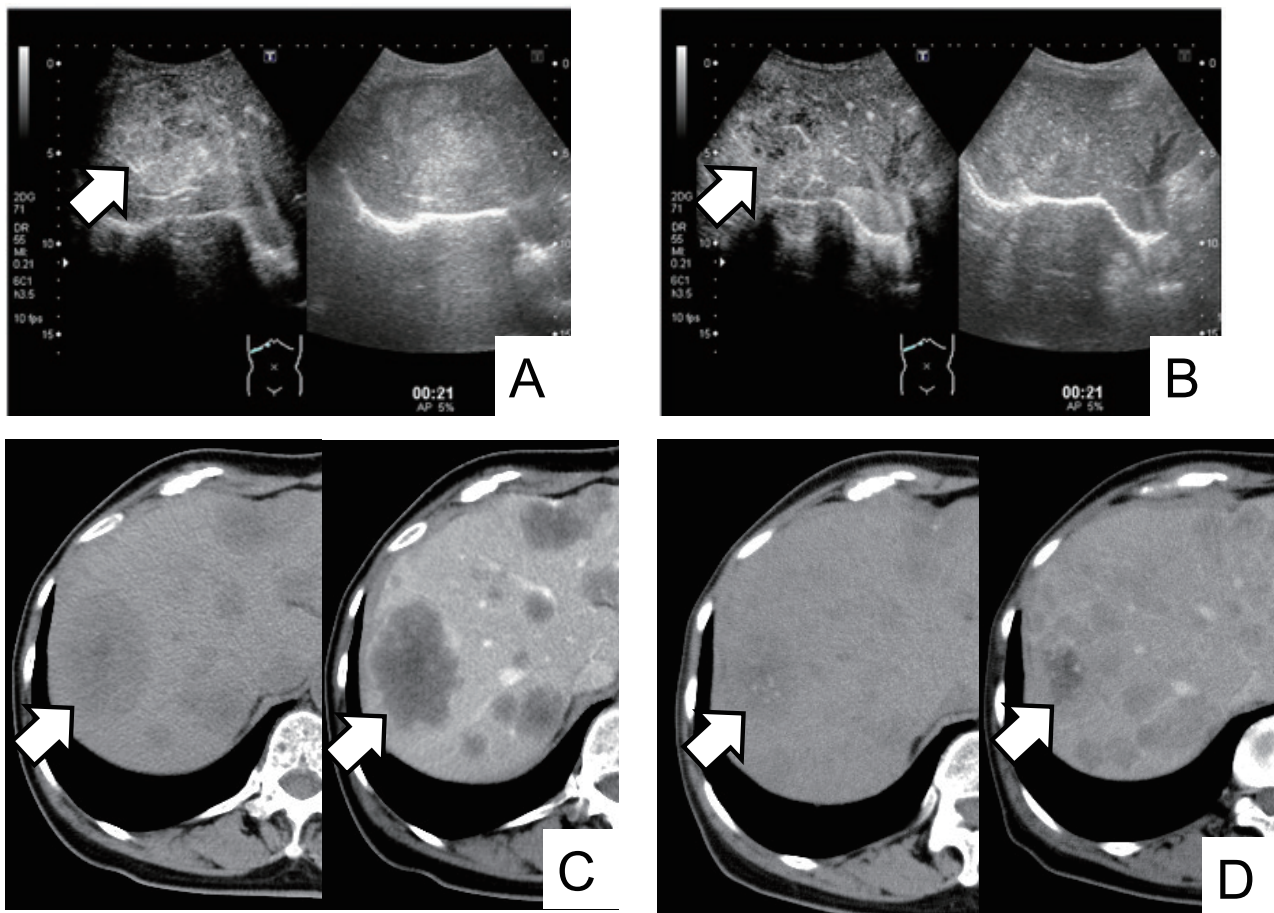


Fig. 3. Several images of a case of partial response to chemotherapy. Arrows indicate the tumor. **A:** CEUS image before chemotherapy. Right image: B-mode. Left image: CEUS mode. Weak contrast enhancement was observed inside the tumor in the arterial phase. **B:** CEUS image after chemotherapy. Right image: B-mode. Left image: CEUS mode. Contrast enhancement was reduced inside the tumor in the arterial phase. **C:** CT image before chemotherapy. Right image: Contrast enhanced CT. Left image: Plain CT. The largest tumor was 57 mm in diameter. **D:** CT image after chemotherapy. Right image: Contrast enhanced CT. Left image: Plain CT. The largest tumor was decreased to 38 mm in diameter. CEUS, contrast-enhanced ultrasonography; CT, computed tomography.

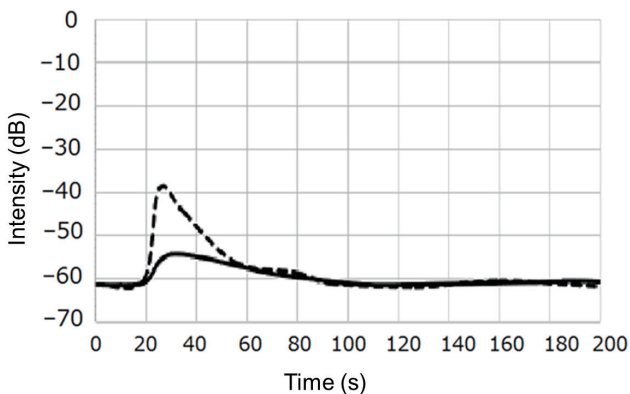


Fig. 4. Graph showing the TIC. Dotted line: TIC before chemotherapy. Solid line: TIC after chemotherapy. The peak intensity, slope of wash-in, and area under the curve were decreased, while the time to wash-in and time to peak identity were unchanged. dB, decibel; TIC, time-intensity curve.

performed after four cycles of FP therapy (cisplatin and fluorouracil). CEUS images showed contrast enhancement of the tumor margins in the arterial phase before chemotherapy and increased contrast enhancement inside the tumor in the arterial phase after chemotherapy. CT images showed an increase in tumor size from 27 to 43 mm (Fig. 5). ROC of the time to wash-in, the slope of wash-in and the area under the curve were increased, while ROC of the peak intensity and the time to peak intensity were decreased (Fig. 6). Two patients of case 2 and 5 underwent CEUS and CT at the same time point (3 and 5 cycle). CT images of case 2 patient showed a reduction in tumor size, and ROC of the peak intensity, the slope of wash-in and the area under the curve were decreased based on CEUS findings. As a patient of case 5, CT images showed an increase in tumor size, and ROC of all parameters of TIC were increased (Table 1).

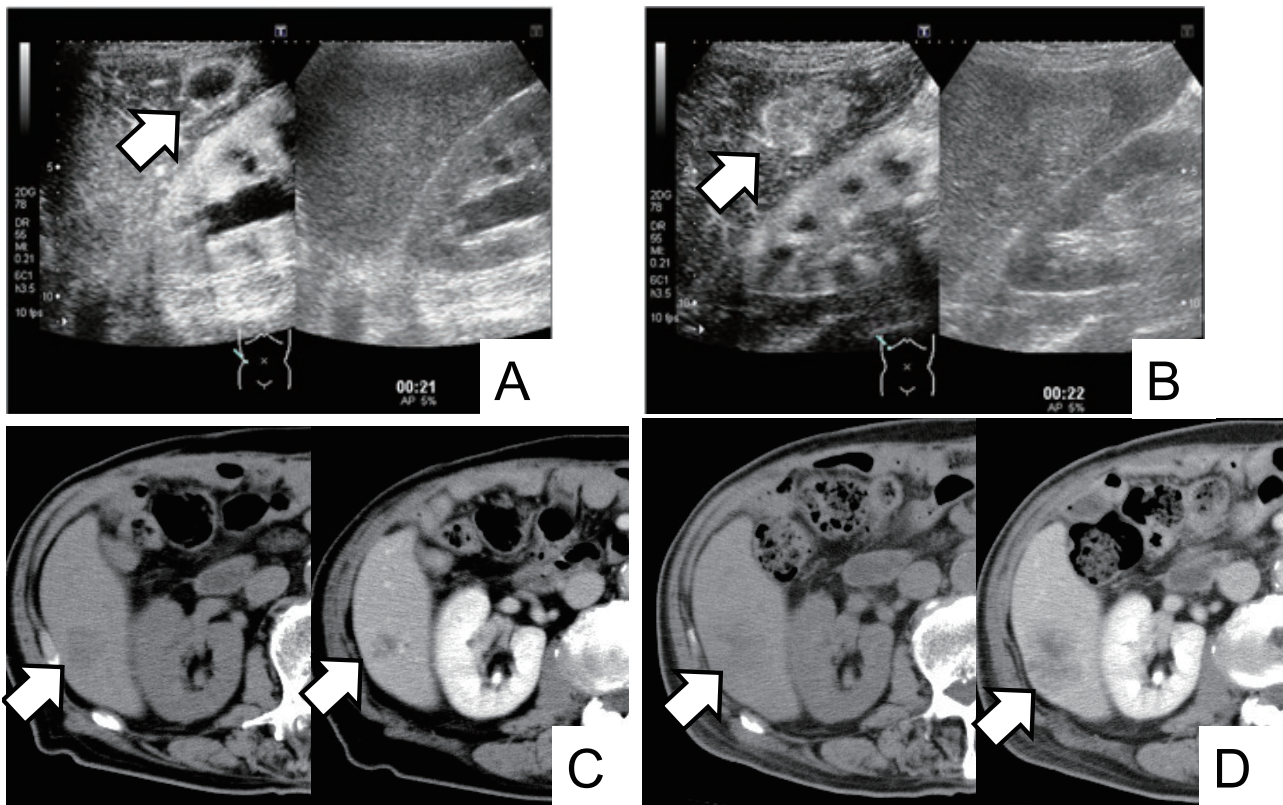


Fig. 5. Several images of a case of progressive disease after chemotherapy. Arrows indicate the tumor. **A:** CEUS image before chemotherapy. Right image: B-mode. Left image: CEUS mode. Contrast enhancement was observed in the tumor margins in the arterial phase. **B:** CEUS image after chemotherapy. Right image: B-mode. Left image: CEUS mode. Contrast enhancement was observed inside the tumor in the arterial phase. **C:** CT image before chemotherapy. Right image: Contrast enhanced CT. Left image: Plain CT. The largest tumor was 27 mm in diameter. **D:** CT image after chemotherapy. Right image: Contrast enhanced CT. Left image: Plain CT. The largest tumor was increased to 43 mm in diameter. CEUS, contrast-enhanced ultrasonography; CT, computed tomography.

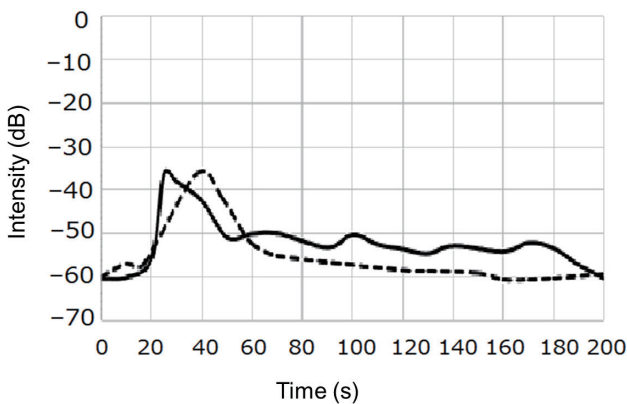


Fig. 6. Graph showing the TIC. Dotted line: TIC before chemotherapy. Solid line: TIC after chemotherapy. The slope of wash-in and area under the curve were increased, while the time to peak intensity was decreased; the peak intensity and time to wash-in were unchanged. dB, decibel; TIC, time-intensity curve.

The findings for all five patients are summarized in Table 1. ROC of the slope of wash-in and the area under the curve, but not in the other TIC parameters, reflected the therapeutic effects based on CT findings.

DISCUSSION

The RECIST, which are based on changes in tumor diameter on CT images, are commonly used to evaluate the therapeutic effect of chemotherapy for liver metastases.⁵ However, the presence of necrosis in the tumor complicates the evaluation, and a more accurate approach would be visualization of tumor hemodynamics. CEUS is a highly sensitive and specific method for detecting liver metastases and provides valuable information about tumor hemodynamics and morphology.⁶⁻¹¹

When a tumor grows or shrinks, feeding vessels are created or damaged, respectively, and morphological changes then become prominent. CEUS reportedly allows for visualization of changes in feeding vessels before tumor morphology changes, enabling early evalu-

ation of the therapeutic effect.¹²⁻¹⁴ This early prediction of the therapeutic effect allows alteration of the method or schedule of treatment, thereby avoiding unnecessary administration of anticancer agents. In this study, we performed CEUS prior to CT in three patients and found that CEUS was able to know the therapeutic effect of anticancer agents at an earlier stage than CT in all cases. Furthermore, this study showed that the findings of CEUS performed at the earliest stage of therapy (after one cycle) reflected the results of the CT evaluation, suggesting that CEUS may have a possibility to predict the therapeutic effect at the earliest stage of chemotherapy. Conversely, patients whose CT images indicated a complete response or stable disease were not examined by CEUS, and the timing of CEUS and CT examinations were not controlled in this study. Further studies with more patients and controlled timing of CEUS and CT examinations are needed. Additionally, this study examined liver metastases from digestive tract cancer, but the type of primary organ was not controlled because there were only five patients included in this study. Liver metastases from digestive tract cancer reportedly show contrast enhancement in the arterial phase.¹⁵ Without well-enhanced contrast in the arterial phase, the peak intensity, time to wash-in and slope of wash-in cannot be accurately calculated. This study indicated that changes in the TIC would be useful in the evaluation of the therapeutic effect when contrast enhancement was present in the arterial phase.

Lassau et al. prepared TICs of CEUS in patients with liver metastases from renal cell carcinoma and found significant differences in the peak intensity, slope of wash-in, time to peak intensity, and area under the curve between groups with and without a therapeutic effect.¹⁶ In this study, ROC of the slope of wash-in and the area under the curve reflected the therapeutic effect, but that of the peak intensity and the time to peak intensity showed no relationship with the therapeutic effect. This may be explained by differences in the locations of primary tumors and by the use of angiogenic inhibitors as anticancer agents in the previous study. Schirin-Sokhan et al. prepared TICs for liver metastases of rectal cancer treated with an angiogenic inhibitor and found that increases in the time to peak intensity were observed when therapy was effective.¹⁴ This suggests that ROC of the time to peak intensity might vary depending on the anticancer agents used. Based on the previous and present studies, it was thought that the angiogenesis of the tumor may reflected the two parameters of TIC (slope of wash in and area under curve). Furthermore, Schirin-Sokhan et al.¹⁴ reported that there were no significant differences in the peak intensity, which is in good agreement with

this study.

In conclusion, the results of this study indicate that in tumors with contrast enhancement in the arterial phase, differences in the TIC between before and after chemotherapy may serve as useful indicators of therapeutic effect for patients with liver metastases. In particular, ROC of the slope of wash-in and the area under the curve appear to specifically reflect the therapeutic effect. Furthermore, there is a possibility that TIC analysis may enable early prediction of the therapeutic effect.

The authors declare no conflict of interest.

REFERENCES

- 1 Frampas E, Lassau N, Zappa M, Vullierme MP, Koscielny S, Vilgrain V. Advanced Hepatocellular Carcinoma: early evaluation of response to targeted therapy and prognostic value of Perfusion CT and Dynamic Contrast Enhanced-Ultrasound. Preliminary results. *Eur J Radiol.* 2013;82:e205-11. PMID: 23273822.
- 2 Lassau N, Bonastre J, Kind M, Vilgrain V, Lacroix J, Cuinet M, et al. Validation of dynamic contrast-enhanced ultrasound in predicting outcomes of antiangiogenic therapy for solid tumors: the French multicenter support for innovative and expensive techniques study. *Invest Radiol.* 2014;49:794-800. PMID: 24570378.
- 3 Lassau N, Chebil M, Chami L, Bidault S, Girard E, Roche A. Dynamic contrast-enhanced ultrasonography (DCE-US): a new tool for the early evaluation of antiangiogenic treatment. *Target Oncol.* 2010;5:53-8. PMID: 20379790.
- 4 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45:228-47. PMID: 19097774.
- 5 Tirumani SH, Kim KW, Nishino M, Howard SA, Krajewski KM, Jagannathan JP, et al. Update on the role of imaging in management of metastatic colorectal cancer. *Radiographics.* 2014;34:1908-28. PMID: 25384292.
- 6 Arita J, Ono Y, Takahashi M, Inoue Y, Takahashi Y, Saiura A. Usefulness of contrast-enhanced intraoperative ultrasound in identifying disappearing liver metastases from colorectal carcinoma after chemotherapy. *Ann Surg Oncol.* 2014;21 Suppl 3:S390-7. PMID: 24570378.
- 7 Mishima M, Toh U, Iwakuma N, Takenaka M, Furukawa M, Akagi Y. Evaluation of contrast Sonazoid-enhanced ultrasonography for the detection of hepatic metastases in breast cancer. *Breast Cancer.* 2016;23:231-41. PMID: 25143060.
- 8 Cantisani V, Grazhdani H, Fioravanti C, Rosignuolo M, Calliada F, Messineo D, et al. Liver metastases: Contrast-enhanced ultrasound compared with computed tomography and magnetic resonance. *World J Gastroenterol.* 2014;20:9998-10007. PMID: 25110428.
- 9 Shiozawa K, Watanabe M, Sumino Y. Evaluation of the hemodynamic status of focal hepatic lesions 20 mm or less in diameter by contrast-enhanced ultrasonography using Sonazoid. *Intervirology.* 2009;52:213-22. PMID: 19556803.
- 10 Yoshida K, Hirokawa T, Moriyasu F, Liu L, Liu GJ, Yamada M, et al. Arterial-phase contrast-enhanced ultrasonography for evaluating anti-angiogenesis treatment: a pilot study. *World J Gastroenterol.* 2011;17:1045-50. PMID: 21448357.

- 11 Lassau N, Paturol-Asselin C, Guinebretiere JM, Leclère J, Koscielny S, Roche A, et al. New hemodynamic approach to angiogenesis: color and pulsed Doppler ultrasonography. *Invest Radiol.* 1999;34:194-8. PMID: 10084663.
- 12 Moschouris H, Malagari K, Marinis A, Kornezos I, Stamatou K, Nikas G, et al. Hepatocellular carcinoma treated with transarterial chemoembolization: Evaluation with parametric contrast-enhanced ultrasonography. *World J Radiol.* 2012;4:379-86. PMID: 22937217.
- 13 Lassau N, Koscielny S, Chami L, Chebil M, Benatsou B, Roche A, et al. Advanced hepatocellular carcinoma: early evaluation of response to bevacizumab therapy at dynamic contrast-enhanced US with quantification--preliminary results. *Radiology.* 2011;258:291-300. PMID: 20980447.
- 14 Schirin-Sokhan R, Winograd R, Roderburg C, Bubenzer J, do Ó NC, Guggenberger D, et al. Response evaluation of chemotherapy in metastatic colorectal cancer by contrast enhanced ultrasound. *World J Gastroenterol.* 2012;18:541-5. PMID: 22363120.
- 15 Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, Nolsøe CP, et al. Guidelines and good clinical practice recommendations for Contrast Enhanced Ultrasound (CEUS) in the liver - update 2012: A WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultrasound Med Biol.* 2013;39:187-210. PMID: 23137926.
- 16 Lassau N, Koscielny S, Albiges L, Chami L, Benatsou B, Chebil M, et al. Metastatic renal cell carcinoma treated with sunitinib: early evaluation of treatment response using dynamic contrast-enhanced ultrasonography. *Clin Cancer Res.* 2010;16:1216-25. PMID: 20145174.