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● *Original Contribution*

UTILITY OF CONTRAST-ENHANCED ULTRASONOGRAPHY WITH PERFLUBUTANE FOR DETERMINING HISTOLOGIC GRADE IN HEPATOCELLULAR CARCINOMA

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Abstract—The purpose of this study was to clarify the diagnostic value of contrast-enhanced ultrasonography (CEUS) with perflubutane in determining the histologic grade in hepatocellular carcinoma (HCC). A total of 147 surgically resected HCCs were dichotomized as well differentiated HCC (wd-HCC) and moderately- or poorly-differentiated HCC (mp-HCC). CEUS findings were evaluated during the arterial phase (vascularity, level and shape of enhancement), portal phase (presence or absence of washout) and post-vascular phase (echo intensity and shape). Receiver operating characteristic (ROC) curve analysis for the diagnosis of mp-HCC yielded area under the ROC curve (A_z) values for arterial phase vascularity and portal phase washout of 0.910 and 0.807, respectively. The A_z value for the combination of vascularity and washout for the diagnosis of mp-HCC was 0.956 (95% confidence interval, 0.910–0.979), corresponding to high diagnostic value. In conclusion, CEUS can provide high-quality imaging assessment for determining the histologic grade of HCCs. (E-mail: tadat0627@gmail.com)

Key Words: **Hepatocellular carcinoma, Histologic grade, Contrast-enhanced ultrasonography.**

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world (El-Serag and Rudolph 2007). With continued surveillance and advances in imaging, the detection rate for localized HCC has increased, resulting in a higher curative surgical resection rate. However, the prognosis of HCC after surgical resection remains poor because of a high rate of recurrence and the lack of effective adjuvant therapy (Llovet et al. 2005). Tumor recurrence occurs in more than 70% of cases at 5 y (Poon 2011), and the 5-y survival rate is between 60% and 70% (Llovet et al. 2005). Histologic grade is one of the predictors of HCC survival and recurrence (Kumada et al. 1997; Tamura et al. 2001). However, biopsy is the only strategy available for obtaining tumor tissue before treatment. Performing a biopsy of HCC traditionally has been avoided because several cases of tumor seeding after biopsy have been reported (Sakurai et al. 1983; Smith 1991). In addition to the risk of seeding, there is also the risk of complications such as bleeding. The assessment of HCC histologic grade using imaging modalities, therefore, can potentially provide valuable information for the management of patients with HCC.

Gray-scale ultrasonography (US) is the most widely used modality for HCC screening and surveillance. However, HCC nodules often have unclear borders, which partly is due to innumerable large regenerating nodules in the cirrhotic liver (Minami et al. 2007). In recent years, because of advancements in US instruments and contrast agents, contrast-enhanced US (CEUS) has been able to demonstrate tumor vascularity with higher sensitivity and accuracy (Furuse et al. 2003; Konopke et al. 2007; Solbiati et al. 2001). Perflubutane (Sonazoid, Daiichi Sankyo, Tokyo, Japan; GE Healthcare, Little Chalfont, UK) is a second-generation US contrast agent composed of a lipid-stabilized suspension of perfluorobutane gas

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microbubbles. Unlike other second-generation contrast agents, perflubutane is phagocytosed by Kupffer cells and it accumulates in the liver parenchyma over time (Yanagisawa et al. 2007). This contrast agent is able to provide information on tumor vascularity as well as high contrast during functional imaging in the post-vascular phase (Korenaga et al. 2009; Ramnarine et al. 2000; Sontum et al. 1999).

In the present study, we clarified the diagnostic impact of histologically grading HCC lesions identified using CEUS with perflubutane. In particular, we analyzed the accuracy of CEUS for distinguishing between well-differentiated HCC (wd-HCC) and moderately- or poorly-differentiated HCC (mp-HCC).

MATERIALS AND METHODS

Patients

Our institution did not require institutional approval or informed consent for review of patient records and images in this retrospective study. Naïve HCC was diagnosed in a total of 552 patients, including 218 (39.5%) patients who underwent surgical resection for treatment of HCC at our institution between January 2007 and December 2013. Of these 218 patients, 147 HCCs in 147 patients were pathologically diagnosed as a single nodular HCC tumor with a maximum diameter of 5 cm or less. Decisions regarding each patient's course of treatment were made based on treatment guidelines for HCC in Japan (Kokudo and Makuuchi 2009). All HCCs were routinely evaluated pre-operatively with CEUS, except in patients allergic to contrast agents. In the present study, all HCCs included in the analysis were evaluated with CEUS within 1 mo of hepatic resection. We excluded patients with multiple HCCs because there was a possibility of insufficient evaluation using CEUS. We also excluded patients with large HCC nodules (diameter >5 cm) because they are often associated with secondary changes that might influence imaging findings such as necrosis, hemorrhage and invasion of portal or hepatic veins.

The patients consisted of 104 men and 43 women with a mean age of 67.4 ± (standard deviation) 9.4 y. There were 28 patients positive for hepatitis B surface antigen (19.0%), 87 positive for hepatitis C virus antibody (59.2%) and 32 negative for both (21.8%). There were 141 Child-Pugh class A patients (95.9%) and 6 class B patients (4.1%) (Pugh et al. 1973). The mean tumor size was 2.4 ± 0.9 cm (Table 1). None of the patients had previously undergone treatment (*e.g.*, ablation therapy, trans-arterial chemoembolization therapy or chemotherapy) for hepatic tumors.

Table 1. Patient and hepatocellular carcinoma characteristics

Age (y)*	67.4 ± 9.4
Sex (male/female)	104/43
Hepatitis virus (B/C/non-B, non-C)	28/87/32
Child-Pugh classification (A/B)	141/6
Tumor size (cm)*	2.4 ± 0.9
Histologic grade (well/moderately/poorly differentiated)	52/86/9
Tumor size according to histologic grade	
wd-HCC (cm)*	2.3 ± 0.9
mp-HCC (cm)*	2.5 ± 0.9

HCC = hepatocellular carcinoma; wd-HCC = well-differentiated HCC; mp-HCC = moderately- or poorly-differentiated HCC.

Of the 147 nodular HCCs, 52 tumors were well differentiated, 86 were moderately differentiated, and 9 were poorly differentiated. Therefore, 52 HCCs were categorized as wd-HCC and the remaining 95 HCCs were categorized as mp-HCC.

* Data expressed as means (±standard deviation).

Contrast-enhanced ultrasonography

Gray-scale sonograms and CEUS images were obtained using an Aplio XG scanner (Toshiba Medical Systems, Tokyo, Japan) with a 5 MHz convex transducer. We used wideband harmonic imaging (referred to commercially as differential tissue harmonic imaging) with transmission and reception frequencies of 1.4 and 5.3 MHz, respectively, as the imaging mode. When a suspicious lesion was identified, CEUS was performed with the focal depth beyond the lesion of interest with a frame rate of 11–15 fps and a dynamic range of 55 dB. A low mechanical index (0.18–0.28) was selected to avoid disruption of the microbubbles. The focal point was just under the bottom of the lesion. Perflubutane with a median microbubble diameter of 2–3 μm was used as the US contrast agent. The recommended clinical dose for the imaging of liver lesions is 0.015 mL encapsulated gas per kilogram of weight. Half of the recommended dose (Maruyama et al. 2009a, 2009b) was administered as a quick bolus and flushed with 10 mL of saline at approximately 1 mL/s *via* a 22-gauge cannula in the antecubital vein.

Tumor enhancement was assessed in the vascular phase (up to 120 s after the injection of contrast agent), whereas parenchymal uptake of the contrast agent was evaluated in the post-vascular phase (starting approximately 10 min after the injection of contrast agent) (Claudon et al. 2013; Terminology and Diagnostic Criteria Committee 2014). Furthermore, during the vascular phase, we defined the arterial phase as up to 30 s after the injection of contrast agent and the portal venous phase as the interval after the arterial phase. The post-vascular images were also called Kupffer images.

Two sonologists from our institution, who had 12 y (K.O.) and 11 y (K.T.) of experience in liver US imaging, participated in this study. Both sonologists had at least 10 y of experience with CEUS of the liver. All CEUS images were recorded on a digital versatile disc (DVD) system for review by two readers who were blinded to the patient's pathologic and clinical data.