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● *Original Contribution*

IRREGULAR DEFECTS IN HEPATOCELLULAR CARCINOMAS DURING THE KUPFFER PHASE OF CONTRAST-ENHANCED ULTRASONOGRAPHY WITH PERFLUOROBUTANE MICROBUBBLES: PATHOLOGICAL FEATURES AND METASTATIC RECURRENCE AFTER SURGICAL RESECTION

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Abstract—The present study aimed to elucidate the association between post-vascular–phase (Kupffer-phase) images from contrast-enhanced ultrasonography (CEUS) with perfluorobutane microbubbles and metastatic recurrences after the resection of hepatocellular carcinoma (HCC). The study examined 73 patients with solitary HCC ≤5 cm in diameter who underwent CEUS before resection. HCC was defined as irregular type (including an irregular defect on Kupffer-phase images) or non-irregular type. Intrahepatic metastatic recurrence was defined as >3 intrahepatic recurrences. Metastatic recurrence included both extrahepatic and intrahepatic recurrences. Frequencies of microscopic portal invasion and intrahepatic metastasis were significantly higher in the irregular group than in the non-irregular group. Cumulative 5-y metastatic recurrence rates in the irregular and non-irregular groups were 43% and 7% ($p = 0.028$), respectively. Multivariate analyses identified Kupffer-phase findings as a factor significantly related to metastatic recurrence. In conclusion, HCCs with an irregular defect during Kupffer-phase CEUS are characterized by more frequent microscopic vascular invasion and intrahepatic metastasis and are significantly associated with metastatic recurrence after resection. (E-mail: tamahide@wakayama-med.ac.jp) © **2017 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Contrast-enhanced ultrasonography, Hepatocellular carcinoma, Resection, Kupffer image, Metastasis, Perfluorobutane microbubble.**

INTRODUCTION

The recurrence rate of hepatocellular carcinoma (HCC) remains extremely high, even after curative treatments such as resection or radiofrequency ablation (RFA). In particular, metastatic recurrence often leads to cancer-related death, because of the difficulty of radical treatment (Shimada et al. 1996). Metastatic recurrences may result from microscopic vascular invasion before treatment.

HCCs with biologically high-grade malignant potential are characterized by a high growth rate, high invasive potential and high metastatic potential. Accordingly, high-grade malignant HCC frequently shows microscopic vascular invasion and/or metastasis that cannot be visualized by imaging before treatment. Some reports have indicated that the gross pathologic type of HCC is strongly associated with histologic differentiation and microscopic vascular invasion, and reflects the malignant potential (Hui et al. 2000; Nakashima et al. 2003; Okusaka et al. 2002; Shimada et al. 2001; Sumie et al. 2008). With the nodular type of HCC, histologic differentiation tends to be poorer in the single nodule with extranodular growth (SNEG) type and contiguous multinodular (CMN) type than in the single nodular (SN) type. The risks of microscopic vascular invasion and intrahepatic metastasis are higher with the SNEG and CMN types than with the SN type. The prognosis with the SNEG or CMN types after resection is thus poorer than with the SN type. Evaluation of gross

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pathologic HCC type by imaging could be important in determining optimal treatment.

Ultrasound currently offers the highest spatial resolution among the imaging modalities used for diagnosing HCCs. B-mode ultrasound may therefore have the potential to accurately evaluate the gross pathologic type. Our previous studies have found that the findings from B-mode ultrasonograms of HCC correlated with histologic differentiation (Moribata et al. 2011) and outcomes after RFA (Moribata et al. 2012). However, as HCCs frequently show unclear margins on B-mode ultrasound, evaluation can be difficult occasionally.

Sonazoid perfluorobutane microbubbles (Daiichi-Sankyo, Tokyo, Japan) represent a second-generation sonographic contrast agent that has been clinically used in Japan since January 2007 and is now also licensed in South Korea. This contrast agent is unique because it accumulates in Kupffer cells, providing persistent and stable enhancement in the post-vascular phase (Kupffer phase) and thus enabling Kupffer imaging (Yanagisawa et al. 2007). During the Kupffer phase, HCC is typically shown as a washed-out defect with high contrast and clear margins. Hatanaka et al. (2010) reported that post-vascular–phase contrast-enhanced ultrasonography (CEUS) with Sonazoid (Daiichi-Sankyo) is a useful tool for evaluating the gross pathologic type of HCC. However, few reports appear to have investigated the utility of Kupffer images from CEUS with Sonazoid (Daiichi-Sankyo) in the evaluation of gross pathologic type. Furthermore, no reports appear to have compared the diagnostic value of HCC gross pathologic type between CEUS and conventional B-mode ultrasonography (US)or analyzed correlations between Kupffer-image patterns and outcomes after resection.

The present study aimed to clarify whether Kupffer-phase images from CEUS with Sonazoid (Daiichi-Sankyo) can predict the gross pathologic type better than conventional B-mode, to identify the clinicopathological features of HCC appearing as an irregular defect during the Kupffer phase and to elucidate the association between Kupffer-phase images and metastatic recurrence after resection.

METHODS

Patients

Using the HCC database of Wakayama Medical University, Tokyo, Japan, 80 patients with HCC (solitary, ≤5 cm in diameter) who underwent Sonazoid ultrasound (Daiichi-Sankyo) within 4 wk before resection were analyzed between April 2007 and November 2012. Excluded from the study were 7 patients who could not be evaluated by CEUS due to poor ultrasound conditions such as a blind spot, deep lesion and/or severe obesity. As a result, we included 73 patients with HCCs in the evaluation of pathologic features. Of these 73, 2 patients died within 3 mo after resection, as a result we analyzed the factors contributing to metastatic recurrence after resection in 71 patients. In all patients, maximum tumor diameter in each case, etiology of hepatitis, Child-Pugh classification and levels of tumor markers such as alpha-fetoprotein (AFP), lens culinaris agglutinin-reactive AFP (AFP-L3) and des-gamma-carboxy prothrombin (DCP) were evaluated before resection. This retrospective study was approved by the ethics committee of Wakayama Medical University and conformed to the Declaration of Helsinki. The need for patients to provide written, informed consent was waived by the ethics committee because of the retrospective design of the study.

Imaging protocols for conventional B-mode US and CEUS

B-mode and CEUS were performed using an ACUSON S2000 with a 4 C1 probe (Siemens Healthcare K.K., Tokyo, Japan) or AplioXV SSA-770A (Toshiba Medical Systems Corporation, Tochigi, Japan) with a 375BT probe (Toshiba Medical Systems). B-mode US was performed using tissue harmonic imaging. CEUS was performed using contrast harmonic imaging mode. A single focus point was set at just beneath the tumor. Acoustic output power was automatically set to a mechanical index value between 0.2 and 0.3, according to the focus point. After an intravenous bolus injection of 0.7 mL of Sonazoid (Daiichi-Sankyo) *via* the antecubital or distal vein, using a 21- or 23-gauge winged needle, tumor vascularity was evaluated during the vascular phase (20–60 s after injection). After vascular-phase images were obtained, ultrasound scanning was stopped until the Kupffer phase. More than 10 min after injection, Kupffer-phase images were obtained by scanning the whole tumor area.

Image analysis of conventional B-mode US and CEUS

Video and fixed images of HCC obtained by conventional B-mode and CEUS were independently analyzed without knowledge of the gross pathologic type or histologic findings by 3 experienced hepatologists, each with 10–20 y of experience of local treatment under ultrasound guidance. Any disagreements in interpretation were resolved by consensus. HCCs were classified into 2 groups according to tumor contours (Nuta et al. 2016). The irregular type was defined as HCC showing an irregular or unclear contour on conventional B-mode or an irregular defect on Kupffer-phase images of CEUS. HCCs not categorized as irregular-type HCC were defined as non-irregular type.