Usefulness of contrast-enhanced ultrasound using perfluorobutane-containing microbubbles as a planning for percutaneous biopsies of focal hepatic lesions: a prospective feasibility study

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Abstract

Aims: To determine whether contrast-enhanced US using perfluorobutane-containing microbubbles (SEUS) would be helpful for planning a hepatic biopsy. **Material and methods:** This prospective study included 40 patients who planned to undergo hepatic biopsy for focal hepatic lesions. All patients underwent B-mode US followed by SEUS. The radiologist evaluated the number of detected lesions, presence of necrosis, conspicuity of target lesion and technical feasibility using 4-point scale. Technical failure and occurrence of change of the target were also assessed. Computer tomography (CT) or magnetic resonance (MR) images were the reference techniques. **Results:** The mean number of lesions detected on CT and MR images was 6.5 ± 8.4 . In 20 (50%) of 40 patients, more focal lesions were detected on SEUS. Targeted lesion was changed in six patients (15%) on SEUS. Mean number of detected lesions on SEUS was significantly higher comparing with B-US (5.1 ± 6.2 vs. 2.8 ± 3.8 , $p < 0.001$). Conspicuity of the targeted lesion was improved in 67.5% (27 of 40) on SEUS and significantly more visualized than B-US (3.6 ± 0.8 vs. 2.8 ± 0.9 , $p < 0.001$). In 7 more patients the necrosis within the lesion was visualized (17.5%) using SEUS. The technical feasibility on SEUS was significantly higher than B-US (2.3 ± 1.0 vs. 3.3 ± 0.9 , $p < 0.001$). Technical failure was observed in only one patient (2.5%). **Conclusions:** SEUS is a helpful technique for planning the hepatic biopsy in terms of detection, improving lesion conspicuity, tumor viable portion assessment and consequently higher operator confidence, compared with B-US.

Keywords: liver; biopsy; neoplasms; ultrasonography; perfluorobutane-containing microbubbles

Introduction

Histopathologic diagnosis is crucial for the management strategy and the optimal treatment planning in patients with focal hepatic lesions [1]. Ultrasonography (US) allows operators to simultaneously observe focal hepatic lesions and to perform percutaneous biopsy of

a lesion in real time. As a result, US-guided biopsy of focal hepatic lesions may be easier and safer to perform than ever before. Nevertheless, US-guided percutaneous biopsy is still an invasive procedure which may cause various complications, including life-threatening bleeding. Therefore, the safety and success rate of percutaneous biopsy of focal hepatic lesions is an important issue and it is essential to obtain accurate and appropriate tissue without complications.

Even though the detection and conspicuity of focal hepatic lesions on US is a key factor determining the success or failure of US-guided percutaneous biopsy [2], not all focal hepatic lesions are easily visible on conventional B-mode US [3]. To overcome the visibility problem of B-mode US, several techniques, including fusion imaging and contrast-enhanced US (CEUS) have been developed [2,4-6]. CEUS utilizes the nonlinear responses from the

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ultrasound contrast microbubbles to enhance tissue [7]. Focal hepatic lesions can be delineated by differences of contrast flow between the lesions and the surrounding hepatic parenchyma [8]. With this mechanism, CEUS increases the lesion-to-liver contrast ratios, and thus making focal hepatic lesions more conspicuous.

There is now a perfluorobutane-containing microbubble contrast agent (Sonazoid, GE Healthcare, Oslo, Norway) consisting of perfluorobutane with a phospholipid shell [9]. Unlike previously introduced ultrasound agents [10,11], Sonazoid allows parenchyma-specific liver imaging, termed Kupffer-phase imaging, as it is taken up by Kupffer cells in the reticuloendothelial system of the liver [12,13]. Malignant lesions that have few or no Kupffer cells can be well-delineated as contrast defects during the Kupffer-phase beginning 10 minutes after administration, while surrounding normal hepatic parenchyma containing normal Kupffer cells appears well-enhanced [14]. Kupffer phase imaging can then be performed for even more than several hours and can provide stable images for longer time and overcome the narrow time window of previously used ultrasound contrast [15].

Several studies regarding the usefulness of CEUS-guided biopsy [4,8,16-18] have been published. However, only a few studies have been reported using perfluorobutane-containing microbubble contrast agent for percutaneous biopsy or radiofrequency ablation (RFA) of focal liver lesions [5,6,19]. Furthermore, there is no published study to assess whether perfluorobutane-containing, microbubbles-enhanced US (SEUS) achieves a change in the biopsy target and helps to determine the appropriate target when planning a biopsy of focal hepatic lesions.

Therefore, the purpose of this study was to determine whether SEUS would be helpful for planning the percutaneous biopsies for focal hepatic lesions and focused on improving lesion detection, tumor viable portion assessment and consequently higher operator confidence, compared with B-mode US.

Materials and methods

This prospective study was approved by our Institutional Review Board, and written informed consent was obtained from all patients before SEUS and the biopsy procedure.

Patients

From September 2016 to February 2017, a total of 332 patients were referred for percutaneous liver biopsy for focal hepatic lesions. The flow diagram of the patient enrollment is summarized in figure 1. Finally, a total of 40 patients (mean age, 57.2 years $\pm$ 11.2 [standard deviation];

Fig 1. Flow diagram of patient population

age range, 21-78 years) were prospectively enrolled in this study. None of the patients had any contraindication for liver biopsy, such as a bleeding tendency. Ten of the 40 patients had a history of chronic liver disease. All the patients included in this study underwent computer tomography (CT) or magnetic resonance (MR) images before the biopsy and had focal hepatic lesions seen on CT (28 cases) or MR images (12 cases). The mean time interval between CT/MRI and the biopsy was 14.5 $\pm$ 16.1 days. The baseline patient demographics are shown in Table I.

Table I. Summary of baseline patient demographics

Characteristic	Value
Age, Mean years $\pm$ SD	57.2 $\pm$ 11.2 (21-78)
Sex, Male:Female	32:8
Past history	
Chronic liver disease	10
Hepatitis B virus	7
Alcohol	1
Cirrhosis of unknown etiology	2
History of malignancy	18
Hepatocellular carcinoma	3
Lung cancer	3
Gastric cancer	2
Colon cancer	1
AOV cancer	1
CBD cancer	1
Breast cancer	1
GIST	2
Renal cell carcinoma	1
Nasopharyngeal cancer	1
Malignant melanoma	1
Neuroendocrine tumor	1
Hepatocellular carcinoma	3
Reference images, CT:MR	28:12

AOV – Ampulla of Vater; CBD – Common Bile Duct; GIST – Gastrointestinal Stromal Tumor; CT – Computer Tomography; MR – Magnetic Resonance

US techniques

US was performed by one of the two board-certified abdominal radiologists (S.M.L. and H.K.Y.) with five to nine years' experience in ultrasound imaging. The examiners were aware of each patient's past medical history and the purpose of this study. A conventional US unit (RS80A, Samsung Medison, Seoul, Korea) with a 1-7 MHz convex probe with a center frequency of 1.3 MHz (CA1-7A, Samsung Medison, Seoul, Korea) was used. The US parameters were as follows: mechanical index of 1.3-1.4, gain of 40-70%, dynamic range of 50 and a frame rate of 26 to 32 pictures/s. Before the US examination, the previous CT or MR images were reviewed and regarded as reference standards. For proper B-mode US examination for the detection of target lesions, parameters including the gain and focal zone were optimized by the operator. Different positions including the intercostal or subcostal approach were applied.

Contrast-enhanced US techniques

Subsequently, SEUS was performed using the same US system by the same operator. The perfluorobutane-containing microbubble contrast agent (Sonazoid, GE Healthcare, Oslo, Norway) was reconstituted with 2 mL of sterile water for injection. One vial of Sonazoid was divided into two doses, each of 1 mL. The first dose was used for pre-biopsy planning and the second dose was used for the targeted biopsy by a manual bolus injection followed by a flush with 10 mL of normal saline via the ante-cubital venous line in each administration. The first dose was used for the detection of focal lesions in the entire liver and the second dose was injected for the target lesion with a fixed single plane. During the second injection the arterial enhancement of the hepatic focal lesion was evaluated. The mechanical index was automatically lowered to 0.198 and the level was adjusted depending on the location and depth of the lesion. Images were obtained at a transmission frequency of 2.4 MHz, a frame rate of 9Hz, a dynamic range of 50, a gain of 63, and a depth of 12-15 cm. SEUS was performed using the asymmetric pulse modulation image mode (PEN2; Samsung Medison, Korea). During the first dose, the liver was continuously scanned during the vascular phase (approximately three minutes) under normal calm breathing and was intermittently scanned until the Kupffer phase (approximately 10-15 minutes). During the first dose the target lesion and the safe route for US-guided biopsy was determined. The images were obtained with both single shot and cine loops and were sent to the Picture Archiving and Communication System (PACS) and were subsequently analyzed by the same operator.

Biopsy procedure

US-guided biopsy was performed by the same radiologists (S.M.L. and H.K.Y.) with experience of >200 cas-

es requiring US-guided biopsy. For the biopsy procedure, the same US system was used for US guidance. The operator chose the target lesion from all the detected lesions and determined the biopsy route considering the SEUS findings in order to avoid vessel injury and achieve adequate tissue sampling. The percutaneous liver biopsy was performed with an 18-gauge, automated, side-cutting biopsy gun (Acecut; TSK Laboratory, Tochigi, Japan) using the free-handle technique. We obtained at least two biopsy specimens and repeated sampling was performed if visual inspection of a specimen was doubtful of the technical success. The number of samplings was determined based on the operator's judgement.

On-site image analysis

The operator conducting the biopsy evaluated the number of lesions, the conspicuity of the target lesion, the presence of necrosis within the target lesion, and the technical feasibility of biopsy on B-mode US and CEUS. The operator evaluated also the number of the focal lesions and the location of the target lesion on the previous CT or MR images. Necrosis within the target lesion was defined as an anechoic area seen on B-mode US and a non-enhancing area during the vascular phase of the SEUS. The conspicuity of the target lesion was scored on a 4-point scale, with a score of 1 indicating definitively non-visualized, score 2 slightly visible and <50% of the lesion with a well-defined margin, score 3 fairly visible and >50% of the lesion with a well-defined margin, and score 4 definitively visible and distinguishable from the surrounding liver parenchyma and >90% of the lesion being visible [20]. The technical feasibility was evaluated depending on the operator's confidence based on a combination of lesion visibility and safety routes, which were graded as: score 1 indicating not feasible due to an invisible lesion or a poorly safe access route; score 2 indicating equivocally feasible due to a partially visible lesion with a fairly safe route; score 3 indicating fairly feasible due to fair lesion conspicuity and a fairly safe access route and score 4 indicating definitively feasible due to good lesion visibility and a good safe access route [21]. Technical failure was considered when SEUS-guided biopsy could not be performed due to non-visualization of the target lesion twice on SEUS or non-visualization of a safe access route. The cases in which the biopsy result was inadequate and needed a re-biopsy were also regarded as a technical failure. Immediate complications were assessed on the spot and delayed complications (<24 hours) were evaluated through the electric medical records. When the pathologic result was positive for neoplasm, the pathologic diagnosis through biopsy was considered as the final diagnosis. Otherwise, the final diagnosis was established based on the patho-

logical examination of the biopsy specimen as well as on the clinical and radiology follow-up.

Statistical analysis

Statistical analyses were performed using commercially available software programs (SPSS, version 23; SPSS, IBM, Armonk, NY, USA; or MedCalc, version 16, MedCalcSoftware, Mariakerke, Belgium). In order to compare the number of detected lesions, the conspicuity of the target lesion, and the subjective technical feasibility on B-mode US and SEUS, a paired T-test was used. The detection rate of necrosis within the target lesion was compared between B-mode US and SEUS using the McNemar test. The chi-square test was used to determine whether detection of necrosis within the lesion was or was not associated with change of the target lesion and the target portion. To determine whether the number of detected lesions on B-mode US and SEUS differed from those of the reference image, a pair-wise comparison with the paired t-test was used. $p < 0.05$ was considered to indicate statistical significance. All numerical data were expressed as mean values $\pm$ standard deviations (SD).

Results

Technical failure on SEUS as well as on B-mode US was observed in one patient whose target lesion was located in the subdiaphragmatic area and was non-visualized. In that case, percutaneous biopsy was appropriately performed with the addition of fusion imaging. No patient showed an inappropriate diagnosis and required re-biopsy. Thirty-three of the 40 patients (82.5%) had a definitive pathologic diagnosis through biopsy: 28 malignant neoplasms (hepatocellular carcinoma [n=8], intrahepatic cholangiocarcinoma [n=3] and hepatic metastasis [n=17]) and 5 benign neoplasms (focal nodular hyperplasia [n=3], cavernous hemangioma [n=1] and peliosis hepatitis [n=1]). The remaining seven patients had no definitive pathologic diagnosis through biopsy; however, their pathology results were helpful for making the final

diagnosis (focal steatosis [n=2], inflammation [n=2], and neutrophilic cholangitis [n=1]) after clinical and imaging follow-up for 8 to 30 weeks. No major, procedure-related complications requiring additional treatment or hospitalization occurred. One patient showed a mild hypersensitivity reaction immediately after contrast injection and recovered after a short amount of time. The mean size of the target lesion measured on the CT and MR images was 3.2 ± 2.2 cm. The target lesions were distributed in segment 2 (n=1), segment 3 (n=2), segment 4 (n=6), segment 5 (n=15), segment 6 (n=3), segment 7 (n=4) and segment 8 (n=9).

The mean number of detected lesions on CT and MR images was 6.5 ± 8.4 . In 20 (50%) of the 40 patients, more focal lesions were detected on SEUS than on B-mode US. The results of the B-mode US and SEUS are detailed in Table II. While the number of detected lesions on B-mode US was significantly lower than that on the CT and MR images ($p < 0.001$), the number on SEUS detected lesions was comparable to that on the CT and MR images ($p = 0.034$). In addition, in 5 out of 40 patients (12.5%) SEUS showed more lesions than the CT/MR images.

The conspicuity of target lesions was improved in 67.5% (27 of 40 patients) using SEUS. Although more than one lesion was visible in 35 patients, focal hepatic lesions detected on CT/MR were not visualized in 5 of the 40 patients on B-mode US. In 4 of these 5 patients, the lesions were visualized on SEUS (fig 2) and the US guided biopsy was performed using the dual split screen mode with B-mode and Kupffer phase image of SEUS.

The conspicuity of the target lesion on SEUS was significantly improved ($p < 0.001$). The detection rate of necrosis within a lesion differed significantly between B-mode US and SEUS ($p = 0.016$; fig 3). Regarding the procedure safety and proper tissue sampling, the target lesion determined on B-mode US was changed in six of the 40 patients (15%) on SEUS and the target portion expected on B-mode US was also changed in nine of the 40 patients (22.5%) on SEUS. The change of the target portion was significantly associated with the additional detection of necrosis within the lesion on SEUS ($p < 0.001$). In 7 patients in whom the necrosis within the lesion was more visualized on SEUS, the target portion was changed after SEUS in 5 patients and the target lesion was changed in 2 patients. The technical feasibility on SEUS was significantly improved ($p < 0.001$) in 32 (80.0%) of the 40 lesions.

Discussions

Our results demonstrate that SEUS increased lesion detection, improved lesion conspicuity and improved vis-

Table II. Comparison of the results of B-mode ultrasonography and SEUS as a planning for percutaneous biopsies of focal hepatic lesions

	B-mode US	SEUS	p value
Number of detected lesions	2.8 $\pm$ 3.8	5.1 $\pm$ 6.2	<0.001
Conspicuity of the target lesion	2.8 $\pm$ 0.9	3.6 $\pm$ 0.8	<0.001
Necrosis within the target lesion	4	11	p=0.016
Technical feasibility	2.3 $\pm$ 1.0	3.3 $\pm$ 0.9	<0.001

The results are expressed as number or mean $\pm$ standard deviation

Fig 2. Hepatic metastasis from melanoma in a 37-year-old man: a) pre-contrast T1WI shows a hyperintense lesion in segment 8; b) Split-screen of SEUS during Kupffer phase (left) and B-mode US (right) show a focal hypoechoic lesion (arrow) on SEUS. However, this lesion was invisible on B-mode US.

Fig 3. Hepatocellular carcinoma in a 60-year-old man: a) contrast enhanced CT during arterial phase shows an enhancing mass with necrosis (asterisk) in right lobe of the liver; b) split-screen of SEUS during vascular phase (left) and B-mode US (right) show an enhancing mass with necrosis (asterisk). However, B-mode US could not delineate necrotic portion within the mass. The percutaneous biopsy for non-necrotic portion could be performed under the guidance of SEUS and pathologic examination revealed hepatocellular carcinoma.

ualization of necrosis within a lesion, compared with B-mode US. Therefore, the target lesion determined by the operator on B-mode US was changed in 6 out of 40 patients (15%) on SEUS and the target portion expected on B-mode US was also changed in 9 out of 40 patients (22.5%) on SEUS. In addition, the technical feasibility improved while performing B-mode US followed by SEUS for biopsy planning, and thus yielding a high technical success rate of 97.5% (39/40).

According to the previous study comparing B-mode US and SEUS including the vascular phase and Kupffer phase, the lesion conspicuity score was significantly better on Kupffer-phase (4.36 ± 1.18) than on vascular phase (3.97 ± 1.41) and B-mode US (3.29 ± 1.25) [5]. Kang et al

reported that the lesion conspicuity in 15 of 16 lesions (93.8%) was increased after adding SEUS, as compared to that on fusion imaging [6]. In our study, the conspicuity of the target lesion on SEUS was significantly improved in 67.5% lesions, results that are consistent with the previous published studies [5,6]. The number of detected lesions in SEUS was slightly lower than that of CT/MR images, but in five patients (12.5%) SEUS showed more lesions than CT/MR images, i.e. the reference images. Mishima et al found also that SEUS has better detection for small hepatic metastasis compared with CT [22].

A previous, large-scale study regarding US-guided biopsy of focal hepatic lesions reported that all biopsy specimens showing insufficiency for diagnosis were

caused by tissue necrosis with poorly preserved cell structure [23]. According to Bang et al, CEUS using Levovist (Schering AG, Berlin, Germany) identified the viable portion in necrotic masses, consequently obtaining adequate specimens [24]. Yoon et al reported that CEUS using SonoVue (Braccon, Milan, Italy) visualized the non-necrotic portion within a necrotic hepatic lesion in one of 44 cases included in the study and therefore successful biopsy was possible [4]. We also found that SEUS had a higher detection rate of necrosis within the lesion than B-mode US and this observation was significantly associated with change of the target portion. To the best of our knowledge, this is the first study which statistically proved that SEUS improved the detection of necrosis within a lesion allowing the target portion to be properly selected.

In our study, technical failure was observed in one patient (1/40, 2.5%) whose target lesion was located in the subdiaphragmatic area and was non-visualized not only on B-mode US but also on SEUS. The subdiaphragmatic location was known as a blind spot on both B-mode US and CEUS [25,26]. In this case with the aid of fusion imaging, percutaneous biopsy was properly performed. Fusion imaging has been used as a guidance tool for biopsy and radiofrequency ablation of focal hepatic lesions, the technique being able to increase the lesion conspicuity and technical feasibility, resulting in a high technical success rate [2,21,27]. However, Lee et al reported that 13.1% (13/99) of small hepatocellular carcinomas (HCCs) between 1 cm and 2 cm in size were still invisible on planning US for RFA with the addition of fusion imaging [28]. Considering the limitation of fusion imaging, Min et al evaluated the usefulness of SEUS in addition to fusion imaging and demonstrated that 83.3% (25/30) of small HCCs that are inconspicuous on fusion imaging with B-mode US became conspicuous on fusion imaging with SEUS [29]. Therefore, fusion imaging and SEUS could be regarded as mutually complementary processes to the guidance for biopsy or RFA of focal hepatic lesions. Further studies comparing SEUS guidance and fusion guidance and determining the indications for each guidance tool on biopsy or RFA of focal hepatic lesions could be interesting.

Our study showed a high technical success rate (97.5%), the results being quite similar to Eso et al [19]. The authors had 92.3% technical success rate of SEUS-guided biopsy for focal hepatic lesions, significantly higher comparing with B-mode US-guided biopsy (76.8%). This could be explained by the fact that SEUS increased the detection and conspicuity of focal hepatic lesions and visualized the viable area within large necrotic lesions, and thus facilitating adequate targeting and sufficient

sampling. Sonazoid might be useful for guidance of focal hepatic lesions in biopsy because of the long duration and high lesion contrast of the Kupffer phase.

Until now there has been no study comparing CEUS using SonoVue and Sonazoid as a guidance tool of hepatic biopsy. SonoVue has been introduced earlier and approved in more countries than Sonazoid [26]. However, Sonazoid is taken up by Kupffer cells, facilitating the persistent enhancement of normal liver parenchyma after vascular phase [12,13]. The Kupffer phase begins 10 min after injection and lasts for an hour or more, while vascular phase is limited to 4-6 min [26]. In our study, for four patients with focal hepatic lesions not-detected on B-mode US, we underwent US guided biopsy using the dual split screen mode of B-mode and Kupffer phase image of SEUS. The long duration of the Sonazoid could take more advantage of real-time biopsy or RFA procedure, especially in the case of HCC [5,6,15,29]. Furthermore, Sonazoid facilitates diagnosis and grading of HCC in patients with a high risk for HCC [14,30,31] and a small viable HCC portion can be depicted using defect-reperfusion imaging by re-injection of Sonazoid [32]. Considering these points, Sonazoid may be a comparable or better guidance tool for biopsy than SonoVue but comparative studies are necessary.

Our study has several limitations. First, even though it was a prospective study, it was only a preliminary study due to the small size of enrolled patients. Therefore, a large scale, multicenter study is needed in order to generalize our hypothesis. Second, on-site image analysis either on B-mode US or on SEUS was evaluated by a single radiologist, and which might, therefore, be somewhat subjective. To overcome this, we strictly defined the grading criteria referring to previous studies [20,21]. Third, we included variable-sized hepatic lesions. In large-sized masses it might be easier to perform percutaneous liver biopsy using B-mode US as well as SEUS. Nevertheless, we demonstrated that the technical feasibility for biopsy as well as lesion detection and the conspicuity on SEUS significantly improved compared with that of B-mode US.

In **conclusion**, SEUS would be helpful for planning the percutaneous biopsy of focal hepatic lesions in terms of the detection, improving lesion conspicuity, tumor viable portion assessment and consequently higher operator confidence, compared with B-mode US.

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