Eur Radiol (2010) 20: 2047–2056
DOI 10.1007/s00330-010-1757-z

ULTRASOUND

Soon Ho Yoon
Kyoung Ho Lee
So Yeon Kim
Young Hoon Kim
Jee Hyun Kim
Sang Hyub Lee
Tae Kyoung Kim

Real-time contrast-enhanced ultrasound-guided biopsy of focal hepatic lesions not localised on B-mode ultrasound

Received: 9 November 2009
Revised: 26 December 2009
Accepted: 4 February 2010
Published online: 23 March 2010
© European Society of Radiology 2010

Electronic supplementary material The online version of this article (doi:10.1007/s00330-010-1757-z) contains supplementary material, which is available to authorized users.

S. H. Yoon · K. H. Lee (✉) ·
S. Y. Kim · Y. H. Kim
Department of Radiology,
Seoul National University Bundang Hospital, Seoul National University College of Medicine,
300 Gumi-dong, Bundang-gu,
Seongnam-si, Gyeonggi-do,
463-707, Korea
e-mail: kholee@snubhrad.snu.ac.kr
Tel.: +82-317-877609
Fax: +82-317-874011

S. H. Yoon
e-mail: yshoka@gmail.com

S. Y. Kim
e-mail: soyeonkim@snubhrad.snu.ac.kr

Y. H Kim
e-mail: yhk@snubhrad.snu.ac.kr

J. H. Kim · S. H. Lee
Department of Internal Medicine,
Seoul National University Bundang Hospital, Seoul National University College of Medicine,
Seoul, Korea

J. H. Kim
e-mail: jhkimmd@snu.ac.kr

S. H. Lee
e-mail: gidoctor@snubh.org

T. K. Kim
Department of Medical Imaging,
Toronto General Hospital,
University of Toronto,
Toronto, ON, Canada
e-mail: TaeKyoung.Kim@uhn.on.ca

Abstract *Objective:* To prospectively evaluate the technical feasibility of percutaneous real-time contrast-enhanced ultrasound (CEUS) guided biopsy of focal hepatic lesions that are not confidently localised on B-mode US. *Methods:* The study included 44 patients (mean age, 61.3 years) whose biopsy target could not be confidently localised on B-mode US performed by two independent radiologists. Biopsy was attempted under the guidance of both CEUS and B-mode US simultaneously displayed on a single monitor. Final diagnosis was established based on the pathological examination of the biopsy specimen as well as on clinical and radiological follow-up. *Results:* The size and depth of the target lesions were 18.0±9.0 mm (mean ± SD) and 41.8±17.2 mm respectively. Five patients with negative or indistinct CEUS findings did not undergo biopsy, while 39 patients completed the biopsy. In 38 of the 39 patients, the biopsy result was concordant with the final diagnosis. In the remaining one patient, the biopsy failed to prove metastasis. As there were six cases of technical failure, the technical success rate was 86% (38/44). The sensitivity in diagnosing malignancy was 88% (30/34). *Conclusion:* Real-time CEUS-guided biopsy is technically feasible for hepatic focal lesions that are not confidently localised on B-mode US.

Keywords Contrast-enhanced ultrasound · Harmonic imaging · Biopsy

Introduction

Ultrasound (US) is the most commonly used imaging technique in guiding percutaneous biopsy of focal hepatic lesions. In guiding biopsy, US has advantages of convenience, safety, low cost, and lack of ionising radiation compared with computed tomography (CT) and magnetic resonance imaging (MR) [1]. Confident visualisation of a target lesion is one of the prerequisites for successful and safe US-guided biopsy. However, the target lesions cannot always be visualised on B-mode US. A meta-analysis [2] reported that diagnostic B-mode US has limited per-patient sensitivity [55%; 95% confidence interval (CI), 41–68%] in detecting hepatic metastases from cancers of the gastrointestinal tract. According to a review [3] on diagnostic B-mode US for hepatocellular carcinoma

(HCC), the reported per-lesion sensitivity ranged from 33 to 84%. A recent study [4] reported that tumours could not be visualised on preprocedural planning B-mode US in 30% of the patients referred for percutaneous radiofrequency ablation of HCC.

Contrast-enhanced US (CEUS) allows better visualisation of focal hepatic lesions that cannot be clearly visualised on B-mode US [5, 6]. Specifically, second-generation US contrast agents enable real-time continuous imaging of focal hepatic lesions for several minutes [7]. This technique has been introduced to help in guiding radiofrequency ablation of hepatic tumours [8, 9]. However, not much attention has been paid to the use of CEUS as a method of real-time guidance for biopsy of focal hepatic lesions [10, 11]. To our knowledge, no prospective study has been conducted to assess the usefulness of real-time CEUS guidance for biopsy of focal hepatic lesions.

The purpose of our study was to prospectively evaluate the technical feasibility of percutaneous CEUS-guided biopsy of focal hepatic lesions that are not confidently localised on B-mode US.

Materials and methods

This single-institutional prospective study was approved by our institutional review board. Informed consent was obtained from all patients.

Study sample

Based on a retrospective study by Schlottmann et al. [11] and our preliminary experience in CEUS-guided biopsy in patients not included in our study, the technical success rate, which was the primary endpoint of our study, was considered to be approximately 90%. With this assumption, the required sample size was estimated to be 44 for the determination of the technical success rate with a 95% CI of ±10% [12]. Therefore, a total of 44 patients were finally included in the study. The age ranged from 34 to 86 (mean ± SD, 61.3±11.8) years. There were 27 men (47–82, 63.1± 8.5 years) and 17 women (34–86, 58.5±15.6 years).

The inclusion criteria were (1) patients referred to the Department of Radiology in our institution for US-guided biopsy of a focal hepatic lesion, and (2) patients with a target lesion that could not be confidently localised on B-mode US performed by two independent radiologists. The exclusion criteria were (1) the biopsy was considered risky due to bleeding tendency (platelet count <50,000 per mm^3 or an international normalised ratio >2) or the patient's severe movements, and (2) contraindication for the use of the contrast agent, such as unstable heart disease, pregnancy, or lactation. If the two radiologists, who independently reviewed previous CT and/or MR images and performed B-mode US, agreed that the target lesion was located in a sonographically blind area (e.g., the anterior extreme of the liver dome), then the patient was further excluded from the study because additional CEUS would not be helpful for the depiction of the target lesion. In addition, if preprocedural CEUS findings were typical of HCC or a benign lesion such as haemangioma or focal nodular hyperplasia, and previous CT and/or MR findings were compatible with the CEUS diagnosis, then the patient was further excluded from the study because a biopsy would not be necessary [13, 14].

Radiologists

All preprocedural planning US and biopsy procedures were performed by one of three radiologists, each of whom had experience performing more than 300 cases of US-guided biopsy of focal hepatic lesions, 10–70 cases of diagnostic CEUS, and 3–10 cases of real-time CEUS-guided intervention. All radiologists were aware of the purpose and design of our study.

B-mode US

The patients fasted for at least 8 h. An 18-gauge intravenous catheter was inserted into the left antecubital vein. One of the three radiologists to whom the patient had initially been referred reviewed the medical record, laboratory test results and the previous cross-sectional imaging studies. The radiologist examined the liver in standard B-mode using a 2–5 MHz curvilinear transducer (IU22, Philips Medical Systems, Bothell, WA, USA). Other transducers including a 5–12 MHz linear transducer were used as needed. Efforts were made to obtain the clearest visualisation of the target lesion by optimising instrument settings, including field of view, gain, placement of the focal zones, insonating frequency and the optional use of the harmonic imaging mode. If a patient had two or more potential target lesions evident from the previous images, the radiologist chose one of the lesions as the biopsy target, taking into consideration the lesion visibility and technical difficulty of the biopsy.

If any target lesion was confidently localised on B-mode US, the biopsy procedure was carried out under the guidance of B-mode US. If the radiologist could not confidently localise any target lesion, then the patient was immediately referred to one of the remaining two radiologists, who performed the same procedure as the first radiologist.

If the second radiologist could not confidently localise a target lesion on B-mode US either, the first and second radiologists were asked to independently complete a questionnaire (Appendix 1), which addressed the visibility of any target lesion (categorised into invisible or visible) and the specific reason for the localisation failure. If the two radiologists consistently considered that the biopsy target was located in a sonographically blind area, the patient was

excluded from the study. The remaining patients, whose target lesions could not be confidently localised on B-mode US by the two independent radiologists, were enrolled in our study.

CEUS

One of the three radiologists performed preprocedural planning CEUS and CEUS-guided biopsy. The clinical purpose of biopsy and the patient body mass index (BMI) were recorded.

We used sulphur hexafluoride microbubbles (Sonovue; Bracco, Milan, Italy), a second-generation contrast agent that is licensed for use in abdominal and vascular imaging in our country. A vial of the contrast agent was divided into two doses of 2.4 mL each. Typically, the first dose was injected intravenously for the preprocedural planning CEUS and the second dose was used for the CEUS-guided biopsy. Each dose was immediately followed by a 10 mL normal saline flush. Using the 2–5 MHz transducer, imaging was performed in a split-screen mode, which displays the CEUS image on the left side and the background B-mode US image on the right side, simultaneously, on a single monitor. Contrast-specific software was used. The mechanical index was 0.06. Focus was positioned at the bottom of the screen to minimise microbubble destruction. Field of view and gain were optimised to provide the clearest depiction of the lesion.

Immediately after the injection, the radiologist searched for the target lesion by continuously sweeping the transducer across the hepatic region considered to contain the target lesion. If no target lesion was identified within 5 min, the previously injected microbubbles were disrupted by imaging the liver in high mechanical index "flash" mode for several minutes, and then the planning CEUS was repeated with the second dose to search for the predetermined target or any other potential target in the liver. If the second planning CEUS did not reveal any target lesion either, then the biopsy was not performed and the location, diameter and depth (from the skin surface to the superficial border of the lesion) of the target lesion were measured from the previous CT or MR image. Such cases were counted as technical failures. However when a target lesion was localised on CEUS, the radiologist could assess the location of the target lesion with reference to surrounding intrahepatic anatomical landmarks, understand the dynamic enhancement pattern of the lesion, measure the diameter and depth of the lesion, plan a safe needle trajectory, and rehearse the biopsy procedure, including the instruction to the patient to suspend respiration.

Biopsy procedure

The skin was sterilised, and 5–10 mL of 2% lidocaine was applied. When the lesion began to clearly appear following the contrast agent injection, an 18-gauge automated side-cutting biopsy needle (Acecut; TSK Laboratory, Tochigi, Japan) was advanced, via either an intercostal or a subcostal approach, using a free-hand technique. The patient was instructed to suspend respiration as the needle punctured through the liver capsule, after which the patient was allowed to breathe shallowly to ensure sufficient time for accurate positioning of the needle. By comparing the needle location on the B-mode US and the target lesion at the CEUS in the split-screen display, the radiologist passed the needle through the target lesion. If the specimen obtained was considered inadequate in a gross inspection by the radiologist, additional biopsy was performed. The number of samplings and any technical difficulty, if present, were recorded.

A radiological technologist recorded the procedure time, which was defined as the time from the initial injection of the microbubbles for the planning CEUS to the procedure termination by the radiologist. The procedure time included the time required for the measurements. Any complications related to the biopsy or the use of the contrast agent were recorded for 24 h following the procedure.

Final diagnosis

If the pathological examination of the specimen revealed findings specific to a neoplasm, the neoplasm was regarded as the final diagnosis. Otherwise, the final diagnosis was established by a study coordinator based on the biopsy results as well as a follow-up of clinical, radiological, laboratory and pathological findings for at least 6 months.

Statistical analysis

The agreement for the B-mode US findings between the two sessions of the B-mode US was assessed with the kappa statistic and concordance rate. The technical feasibility of CEUS-guided biopsy was assessed with two descriptive statistics with Clopper-Pearson 95% CIs: technical success rate and sensitivity in diagnosing malignancy. The technical success rate was defined as the number of technical successes divided by the number of patients included. A case was counted as a technical success if the pathological findings of the specimen specifically represented the final diagnosis. A case was counted as a technical failure if the patient did not complete the biopsy procedure for any reason or if the pathological findings of the specimen did not specifically represent the final diagnosis. In diagnosing malignancy, the biopsy result was considered as a true-positive if the pathological findings revealed a malignancy and as a false-negative if the biopsy procedure was a technical failure and the final diagnosis was a malignancy. Because of the small sample

size, we could not perform subgroup analyses across radiologists or lesion characteristics including the location, size and depth.

Results

Patient inclusion

Between June 2008 and June 2009, 538 patients were referred for US-guided biopsy of focal hepatic lesions. Seven of these patients did not undergo biopsy because of a bleeding tendency. Of the remainder, 478 patients underwent biopsy under B-mode US guidance following the first (188, 152 and 127 patients by radiologists 1, 2 and 3 respectively) or second (6, 3 and 2 patients by radiologists 1, 2 and 3 respectively) B-mode US.

In the remaining 53 patients, the target lesion could not be confidently localised on the B-mode US performed by two independent radiologists. Seven of these patients were excluded because the two radiologists agreed that the target lesion was located in a sonographically blind area (Table 1). The remaining 46 patients underwent CEUS. Two of these were further excluded, as the target lesion was considered to be benign (abscess and focal nodular hyperplasia respectively) based on the CEUS findings, in addition to the other radiological and clinical findings. These two patients did not undergo biopsy (Fig. 1).

The BMI of the 44 patients finally enrolled was 22.2± 2.9 (mean ± SD) kg/m^2 (Table 2). Based on the findings of CT (n=27), MR (n=1),or both (n=16) obtained 1–28 days before the biopsy, the median number of hepatic focal lesions was one. In 19 patients with two or more lesions, biopsy was performed for only one lesion because there was no clinical or radiological reason to obtain tissue samples from different lesions. The purpose of the biopsy was to diagnose or rule out hepatic metastasis detected at the initial presentation (n=21) or postoperative surveillance (n=14) of a malignancy, or to rule out malignancy for an indeterminate hepatic lesion (n=9).

B-mode US

In 53 patients, the target lesion could not be confidently localised in the first (17, 19 and 17 patients by radiologists 1, 2 and 3, respectively) and second (18, 17 and 18 patients by radiologists 1, 2 and 3, respectively) sessions of B-mode US. Regarding the lesion visibility on the B-mode US, the responses of the two radiologists involved in the B-mode US were concordant in 46 (87%) of the 53 patients (invisible in 20 patients and visible in 26 patients) and discordant in the remaining 7 patients (13%) (kappa = 0.73, p<0.001). In determining the specific reasons for localisation failure, the concordance rate of the two radiologists was 75% (40/53). The two most frequent reasons were an indistinct lesion on the B-mode US, making it difficult to guide a needle or to verify the lesion as the target lesion (Fig. 2), and an invisible target lesion because of isoechogenicity (Fig. 3). Other reasons included the lesion location in a sonographically blind area, inability to identify a non-necrotic portion as an adequate biopsy site within a necrotic lesion (Fig. 4) and a lesion presumably hidden in a heterogeneous hepatic echo area (Table 1).

CEUS

In 5 of the 44 patients finally enrolled, the target lesion was either not identified (n=4) or very indistinct (n=1) on the CEUS following both injections. In the remaining 39 patients (89%), the target lesion clearly appeared hypoechoic from 20–30 s after the injection, providing an excellent lesion-to-liver contrast for several minutes.

Lesion characteristics

Twenty-eight and 16 lesions were located in the right and left liver, respectively. The mean lesion size was 18.0±9.0 (mean ± SD) mm. The mean lesion depth was 41.8± 17.2 mm (Table 3).

Table 1 B-mode ultrasound findings in 53 patients with target lesion not localised on B-mode ultrasound

Lesion visibility and reason for localisation failure	First B-mode ultrasound	Second B-mode ultrasound
Invisible	26 (8)	21 (8)
Isoechoic	16 (1)	13 (1)
Sonographically blind area	10 (7)	8 (7)
Poor ultrasound penetration	0	0
Visible	27 (1)	32 (1)
Very indistinct	22 (1)	28 (1)
Internal necrosis	3	2
Presumable lesion in a heterogeneous echo area	2	2

Data are number of patients. Data *in parentheses* are for the excluded patients as the target lesion was considered to be in a sonographically blind area (n=7) or the lesion was considered benign (n=2) on subsequent contrast-enhanced ultrasound

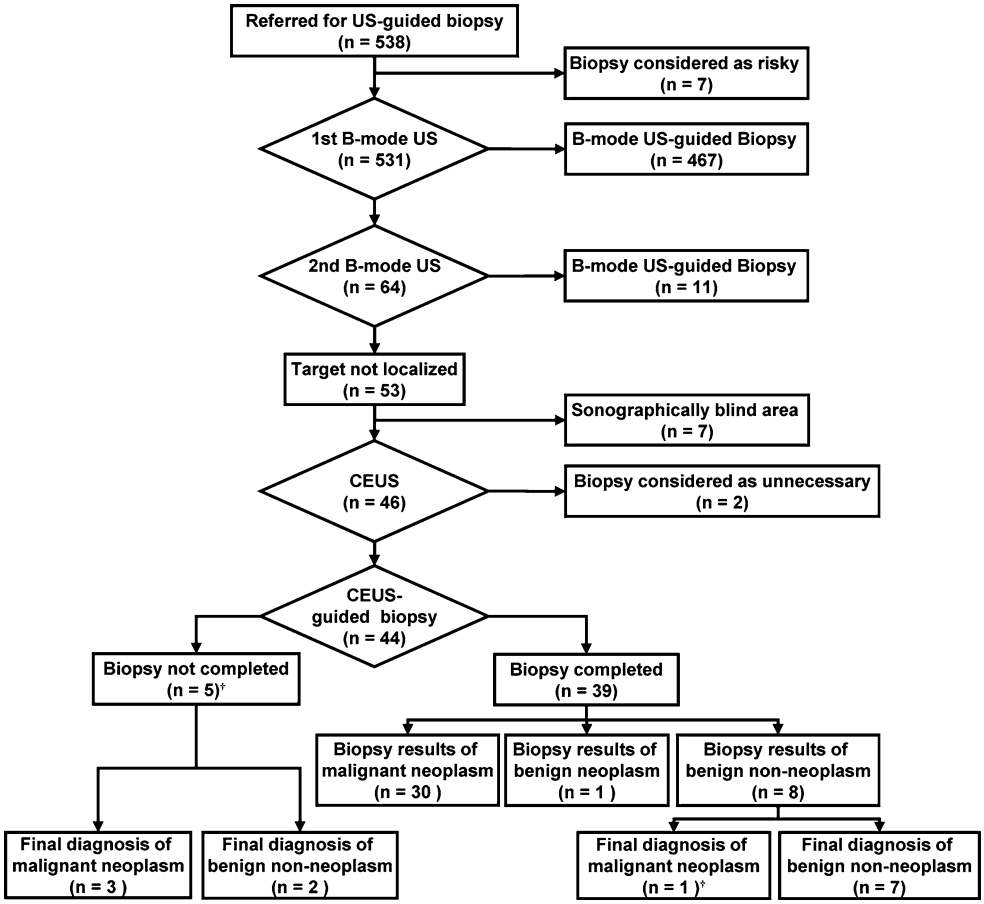

Fig. 1 Patient flow diagram. *CEUS* Contrast-enhanced US, *US* ultrasound. †Technical failures

Biopsy procedure

The five patients with either negative or indistinct CEUS findings did not undergo biopsy. In the remaining 39 patients, biopsy was performed by one of the three radiologists (17, 12, and 10 patients each) for the predetermined target lesion, using the real-time CEUS guidance through an intercostal (n=28) or subcostal (n=11) approach. The number of needle passes was one, two, three and four in 22, 10, 6 and 1 patients, respectively. In the 39 patients whose biopsy procedure was completed, the procedure time ranged from 6.4 to 33.9 (11.5±6.1, mean ± SD) min. In four patients, more than two injections of contrast agent from a new vial were needed to restore the lesion-to-liver contrast that declined as the procedure was prolonged because of the difficulty in appropriate needle placement. Otherwise, there were no technical difficulties. There was no complication other than pain at the needle entry site, and this subsided spontaneously (Table 4).

Table 2 Patient characteristics (n=44)

Patient characteristics	Data
Sex	
Male	27 (3)
Female	17 (3)
Age (years)	
Range	34–86 (38–74)
Mean ± SD	61.3±11.8 (57.8±16.0)
BMI (kg/m^2)	
<18.5	2 (2)
18.5–24.9	32 (4)
≥25.0	10
No. of hepatic focal lesion(s)	
1	25 (4)
2–5	17 (2)
6 or more	2

Unless otherwise indicated, data are number of patients. Data *in parentheses* are for the six technical failure cases

Final diagnosis

Final diagnoses in the 44 patients are listed in Table 5. The pathological examination of the biopsy specimen obtained in 39 patients revealed malignant neoplasm, benign neoplasm and benign non-neoplastic lesion in 30, 1 and 8 patients, respectively. In seven of the eight patients with benign non-neoplastic biopsy results (four cases of eosi-

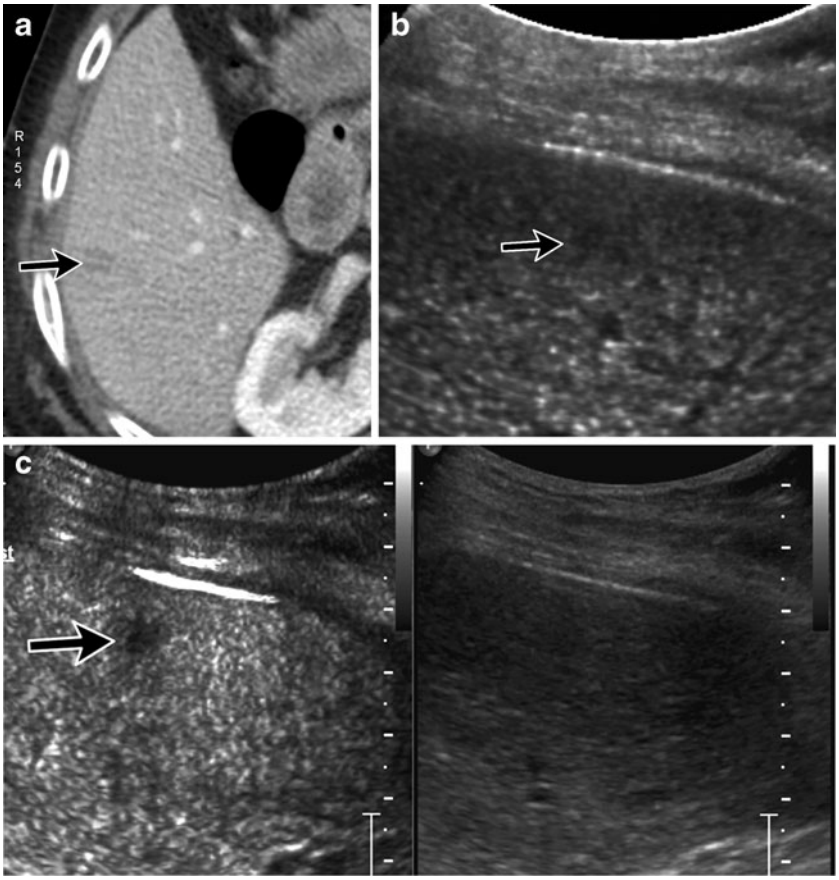

Fig. 2 Hepatic eosinophilic abscess in a 70-year-old man with melanoma. **a** Contrast-enhanced transverse CT shows a subtle hypoattenuating focal lesion (*arrow*) at the segment 6. **b** Oblique intercostal B-mode ultrasound shows an indistinct lesion presumed to be the target lesion. **c** Split-screen display of oblique intercostal ultrasound shows the 9-mm lesion (*arrow*) with increased conspicuity on the contrast-enhanced image (*left*). Biopsy was performed under the real-time guidance of contrast-enhanced ultrasound (Supplemental Movie 1)

nophilic abscesses, two cases of abscess at the healing stage and one case of bile sludge with surrounding inflammation), each biopsy result was verified with the clinical and radiological follow-up for 6–11 months. In the remaining one patient who had pancreatic cancer, the biopsy result was necrotic and fibrotic debris only; however, the target lesion was finally determined as metastasis based on the follow-up CT, which showed interval growth of the target lesion and the development of new hepatic nodules. This patient was counted as a technical failure, as the biopsy result was considered inadequate.

In three of the five patients who did not undergo biopsy, the final diagnosis of the target lesion was determined as

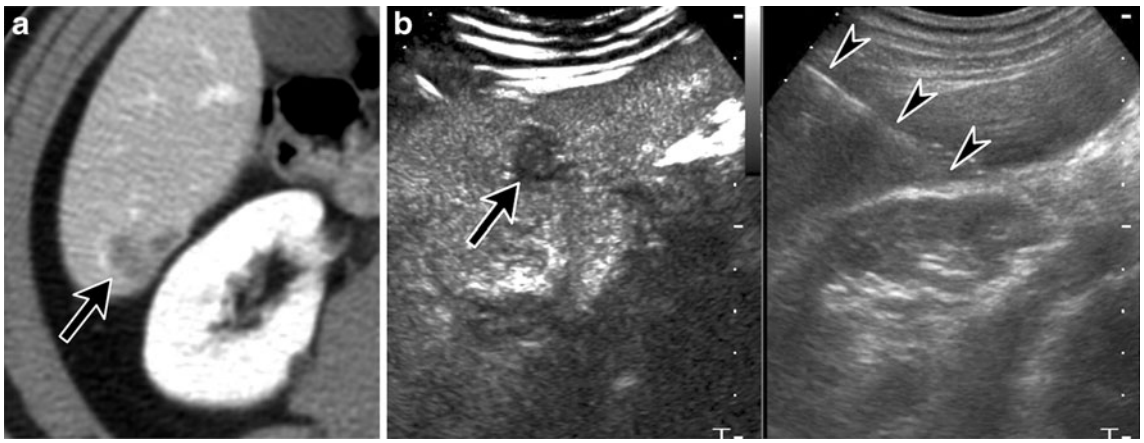

Fig. 3 Hepatic metastasis from glottic cancer in a 57-year-old man. **a** Contrast-enhanced transverse CT shows a hypoattenuating focal lesion (*arrow*) at the segment 6. The target lesion was invisible on B-mode ultrasound (Supplemental Movie 2). **b** Split-screen display of oblique intercostal ultrasound clearly depicts the 14 mm lesion (*arrow*) on the contrast-enhanced image (*left*) and the biopsy needle (*arrowheads*) on the B-mode image (*right*)

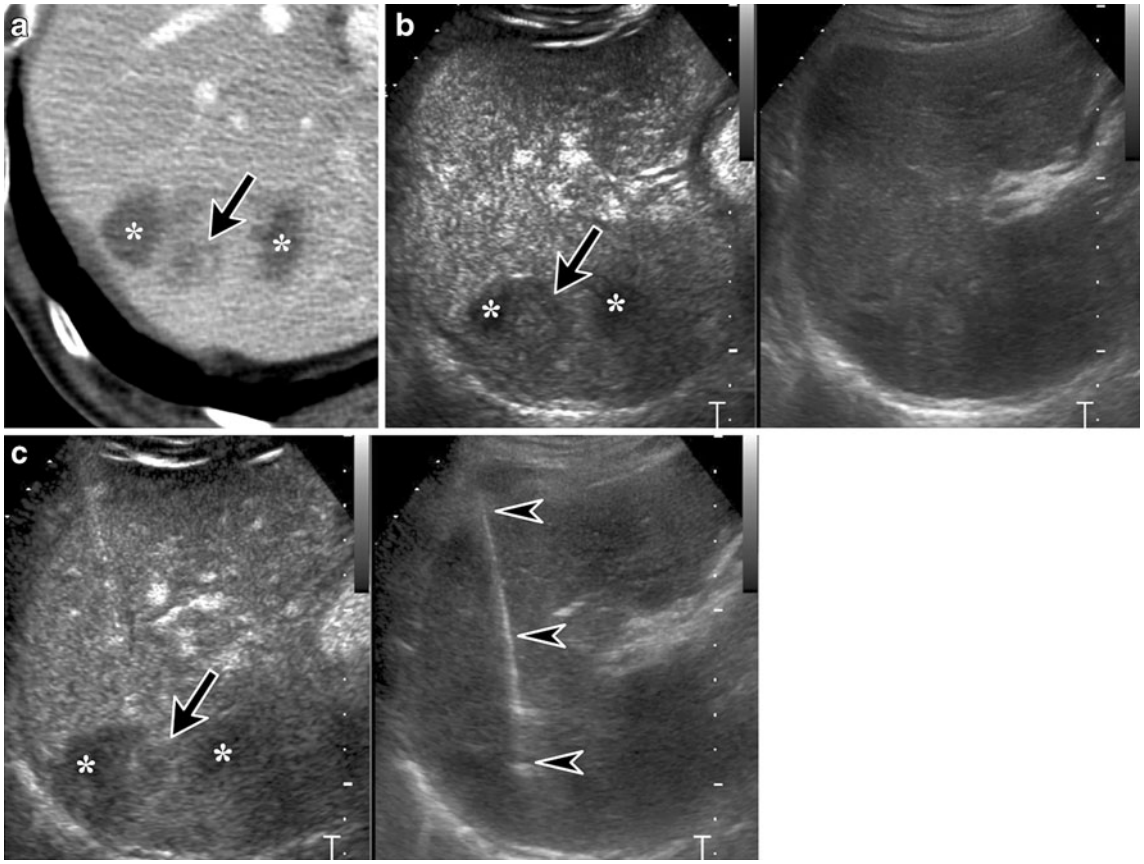

Fig. 4 Marginal recurrence following radiofrequency ablation of hepatic metastases from gastric cancer in a 72-year-old man. **a** Contrast-enhanced transverse CT shows a suspicious viable tumour (*arrow*) around the two previously ablated areas (*asterisks*) at the segment 7. On B-mode ultrasound, the target lesion could not be confidently localised, as the target lesion and the surrounding previously ablated tumours appeared as a single conglomerate mass (Supplemental Movie 3). **b** Split-screen display of transverse subcostal ultrasound during the early enhancement phase clearly depicts the 22 mm target lesion (*arrow*) with contrast enhancement compared with the previously ablated areas (*asterisks*). **c** A biopsy needle (*arrowhead*) was successfully placed for the target lesion (*arrow*)

metastasis, based on the follow-up CT showing the growth of the target lesion. In the remaining two patients, the final diagnosis was considered as focal inflammation (eosinophilic abscess in each patient), based on clinical and radiological follow-up for 6 months.

Technical success rate

As there were 38 technical successes (including the 30 malignant neoplasms, 1 benign neoplasm and 7 benign non-neoplastic lesions) and 6 technical failures (including the 5 patients who did not undergo the biopsy and 1 patient with the inadequate biopsy result), the overall technical success rate was 86% (95% CI, 73–95%; 38/44). The individual technical success rates of radiologists 1, 2 and 3 were 84% (16/19), 92% (12/13) and 83% (10/12) respectively. In diagnosing malignancy, there were 30 true-positives and 4 false-negatives. The overall sensitivity in the diagnosis of malignancy was 88% (95% CI, 73–97%; 30/34), with individual sensitivities of radiologists 1, 2 and 3 calculated as 85% (11/13), 91% (10/11) and 90% (9/10), respectively.

Discussion

In this study, we report our prospective results of real-time CEUS-guided biopsy of focal hepatic lesions in 44 patients whose biopsy target could not be confidently localised on B-mode US. The technical success rate was 86% (38/44) and the sensitivity in diagnosing malignancy was 88% (30/34).

If a biopsy target cannot be localised on B-mode US clearly, an alternative localising method can be used including CT [15], MR [16], laparoscopy or laparotomy to guide the biopsy. Compared with these methods, CEUS guiding has advantages of convenience, availability, safety, low cost and lack of ionising radiation [1]. In addition, preprocedural CEUS enables further characterisation of the

Table 3 Lesion characteristics (n=44)

Lesion characteristics	Data
Location	
Segment 1	1
Segment 2	3
Segment 3	5
Segment 4	7 (2)
Segment 5	10 (1)
Segment 6	9 (2)
Segment 7	4
Segment 8	5 (1)
Diameter (mm)	
Range	5–33 (5–31)
Mean ± SD	18.0±9.0 (12.2±9.5)
Median	16 (9)
Depth (mm)	
Range	19–92 (19–55)
Mean ± SD	41.8±17.2 (31.0±13.8)
Median	36 (28)

Unless otherwise indicated, data are number of patients. Data *in parentheses* are for the six technical failure cases

target lesion to avoid unnecessary biopsy and helps in identifying a non-necrotic portion as an adequate biopsy site within a necrotic lesion, as shown in our cases. Considering all of these advantages, we believe that CEUS will become the method of choice in guiding biopsy in patients whose biopsy target cannot be confidently localised on B-mode US.

Table 4 Biopsy procedure (n=44)

Biopsy procedure	Data
Approach	
Intercostal	28 (1)
Subcostal	11
None	5 (5)
No. of needle passes	
0	5 (5)
1	22 (1)
2	10
3	6
4	1
No. of injection(s) of contrast agent[a]	
2	40 (6)
3	3
4	1
Procedure time (min)	
Range	6.4–33.9 (6.6–18.2)
Mean ± SD	11.7±5.8 (13.2±3.8)
Median	8.7 (13.8)

Unless otherwise indicated, data are number of patients. Data *in parentheses* are for the six technical failure cases
[a]Including the injection for preprocedural planning ultrasound

Table 5 Final diagnosis (n=44)

Final diagnosis	No. of patients
Malignancy	34 (4)
Primary hepatic malignancy	4
Cholangiocarcinomas	3
Hepatocellular carcinoma	1
Metastasis from	30 (4)
Pancreatic cancer	17 (2)
Gallbladder cancer	4
Gastric cancer	3
Colorectal cancer	2 (1)
Bile duct cancer	2
Lung cancer	1 (1)
Glottic cancer	1
Benign	10 (2)
Benign neoplasm	1
Hepatic adenoma	1
Non-neoplastic lesion	9 (2)
Eosinophilic abscess	6 (2)
Abscess at healing stage	2
Bile sludge	1

Data *in parentheses* are for the six technical failure cases

To our knowledge, our study is the first attempt to prospectively assess the usefulness of real-time CEUS guidance for biopsy of focal hepatic lesions. There have been three previous studies on the use of CEUS to aid in the biopsy of hepatic focal lesions. Bang et al. [10] reported the first case in which they used CEUS to identify the non-necrotic portion as an adequate biopsy site within a large neurofibrosarcoma. They slowly injected (1 mL/s) a first-generation contrast agent in order to sustain the contrast enhancement during the biopsy procedure, as the unstable microbubbles were rapidly destroyed with the colour Doppler mode they used. Using sulphur hexafluoride microbubbles, Schlottmann et al. [11] reported a technical success rate of 91% (10/11) in a retrospective analysis of a single operator's results in real-time CEUS-guided biopsy of lesions ranging in size from 7 to 79 mm that were invisible on B-mode US. They used additional software to increase the echo signals from the needle and microbubbles because the original harmonic imaging mode they used yielded unsatisfactory image quality. Using the same contrast agent, Wu et al. [17] reported that the technical success rate of biopsy was significantly improved with preprocedural planning CEUS. In this study, however, the CEUS was performed before the biopsy and only B-mode US was used for the real-time guidance.

In our study, we used the split-screen mode as we found that the conspicuity of the needle was better on the B-mode image than on the CEUS image. We postulate that the bright contrast enhancement in the hepatic parenchyma

masked the echogenic biopsy needle in the CEUS. Although an operator needs to look at the two real-time images (the CEUS and B-mode US), our radiologists were comfortable with this technique. It should be noted that the split-screen mode that we used is proprietary to the manufacturer, although similar display modes are available from other manufacturers. A disadvantage of the split-screen mode that we used is that the B-mode US obtained with low mechanical index has lower image quality compared with a standard B-mode US, although the needle could still be clearly identified.

It might be technically possible for a very highly experienced operator to perform biopsy for very indistinct or even invisible lesions under B-mode guidance only, in a "stereotactic" manner, depending on anatomical landmarks by referring to the previous CT or MR images. However, in our experience, this type of procedure can be associated with inappropriate tissue sampling (and therefore, the negative biopsy results will often be mistrusted) and also with an increased number of needle passes, especially for small lesions.

Our study has limitations. First, the patient selection depended on the subjective confidence of the radiologists in localising the lesion on B-mode US. This limitation was inevitable, as determination of whether a target lesion is clearly localised at guiding US should be decided by the operator in clinical practice. To reduce and measure the subjectivity, we involved two independent radiologists in determining the confidence. Nevertheless, interobserver disagreement still existed, as expected, for both the lesion visibility and for the reason for the localisation failure. Second, many (34/44) of the patients had a BMI less than 25 kg/m^2, which is probably associated with a high prevalence of advanced malignancy in the study sample. The technical success rate might be lower for patients with greater body sizes because of limited ultrasound penetration and the longer needle trajectory. Third, this is a single-institution study using a single type of US equipment and involving only three radiologists with considerable experience in US-guided biopsy. Therefore, our results might be generalised to large centers only. However, it should be noted that the CEUS guidance technique is very straightforward, with no essential difference from B-mode US guidance except for the use of contrast enhancement.

In conclusion, percutaneous real-time CEUS-guided biopsy is technically feasible for hepatic focal lesions that are not confidently localised on B-mode US.

Acknowledgements This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by Ministry of Science and Technology (MOST), Republic of Korea (no. R01–2008–000–10055–0).

Appendix. Questionnaire for B-mode ultrasound findings

If a target lesion was never visible, choose one of the following reasons:

(a) Isoechoic in comparison to the surrounding hepatic parenchyma
(b) Located in a sonographically blind area
(c) Poor ultrasound penetration due to severe fatty liver or large body habitus
(d) Others. Describe _____

If any target lesion was visible with any confidence, choose one of the following reasons:

(e) Too indistinct to guide a needle or to be verified as a true lesion
(f) Unable to discriminate non-necrotic portion from necrotic portion in the lesion
(g) Others. Describe _____

References

1. Bravo AA, Sheth SG, Chopra S (2001) Liver biopsy. N Engl J Med 344:495–500
2. Kinkel K, Lu Y, Both M, Warren RS, Thoeni RF (2002) Detection of hepatic metastases from cancers of the gastrointestinal tract by using noninvasive imaging methods (US, CT, MR Imaging, PET): a meta-analysis. Radiology 224:748–756
3. Teefey SA, Hildeboldt CC, Dehdashti F, Siegel BA, Peters MG, Heiken JP, Brown JJ, McFarland EG, Middleton WD, Balfe DM, Ritter JH (2003) Detection of primary hepatic malignancy in liver transplant candidates: prospective comparison of CT, MR imaging, US, and PET. Radiology 226:533–542
4. Rhim H, Lee MH, Kim YS, Choi D, Lee WJ, Lim HK (2008) Planning sonography to assess the feasibility of percutaneous radiofrequency ablation of hepatocellular carcinomas. Am J Roentgenol 190:1324–1330
5. Albrecht T, Blomley MJ, Burns PN, Wilson S, Harvey CJ, Leen E, Claudon M, Calliada F, Correas JM, LaFortune M, Campani R, Hoffmann CW, Cosgrove DO, LeFevre F (2003) Improved detection of hepatic metastases with pulse-inversion US during the liver-specific phase of SHU 508A: multicenter study. Radiology 227:361–370

6. Chami L, Lassau N, Malka D, Ducreux M, Bidault S, Roche A, Elias D (2008) Benefits of contrast-enhanced sonography for the detection of liver lesions: comparison with histologic findings. Am J Roentgenol 190:683–690
7. Catalano O, Nunziata A, Lobianco R, Siani A (2005) Real-time harmonic contrast material-specific US of focal liver lesions. Radiographics 25:333–349
8. Minami Y, Kudo M, Chung H, Kawasaki T, Yagyu Y, Shimono T, Shiozaki H (2007) Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. Am J Roentgenol 188:489–494
9. Minami Y, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H (2004) Treatment of hepatocellular carcinoma with percutaneous radiofrequency ablation: usefulness of contrast harmonic sonography for lesions poorly defined with B-mode sonography. Am J Roentgenol 183:153–156
10. Bang N, Bachmann Nielsen M, Vejborg I, Mellon Mogensen A (2000) Clinical report: contrast enhancement of tumor perfusion as a guidance for biopsy. Eur J Ultrasound 12:159–161
11. Schlottmann K, Klebl F, Zorger N, Feuerbach S, Scholmerich J (2004) Contrast-enhanced ultrasound allows for interventions of hepatic lesions which are invisible on conventional B-mode. Z Gastroenterol 42:303–310
12. Newcombe RG (1998) Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med 17:857–872
13. Bruix J, Sherman M (2005) Management of hepatocellular carcinoma. Hepatology 42:1208–1236
14. Lencioni R (2006) Impact of European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines on the use of contrast agents in liver ultrasound. Eur Radiol 16:1610–1613
15. Chojniak R, Isberner RK, Viana LM, Yu LS, Aita AA, Soares FA (2006) Computed tomography guided needle biopsy: experience from 1,300 procedures. Sao Paulo Med J 124:10–14
16. Stattaus J, Maderwald S, Baba HA, Gerken G, Barkhausen J, Forsting M, Ladd ME (2008) MR-guided liver biopsy within a short, wide-bore 1.5 Tesla MR system. Eur Radiol 18:2865–2873
17. Wu W, Chen MH, Yin SS, Yan K, Fan ZH, Yang W, Dai Y, Huo L, Li JY (2006) The role of contrast-enhanced sonography of focal liver lesions before percutaneous biopsy. Am J Roentgenol 187:752–761