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Sonazoid™ versus SonoVue® for Diagnosing Hepatocellular Carcinoma Using Contrast-Enhanced Ultrasound in At-Risk Individuals: A Prospective, Single-Center, Intraindividual, Noninferiority Study

Hyo-Jin Kang[1, 2], Jeong Min Lee[1, 2, 3], Jeong Hee Yoon[1, 2], Jeongin Yoo[1, 2], Yunhee Choi[4], Ijin Joo[1, 2, 3], Joon Koo Han[1, 2, 3]

[1]Department of Radiology, Seoul National University Hospital, Seoul, Korea; [2]Department of Radiology, Seoul National University College of Medicine, Seoul, Korea; [3]Institute of Radiation Medicine, Seoul National University Medical Research Center, Seoul, Korea; [4]Division of Medical Statistics, Medical Research Collaborating Center, Seoul National University Hospital, Seoul, Korea

Objective: To determine whether Sonazoid-enhanced ultrasound (SZUS) was noninferior to SonoVue-enhanced ultrasound (SVUS) in diagnosing hepatocellular carcinoma (HCC) using the same diagnostic criteria.

Materials and Methods: This prospective, single-center, noninferiority study (NCT04847726) enrolled 105 at-risk participants (71 male; mean age ± standard deviation, 63 ± 11 years; range, 26–86 years) with treatment-naïve solid hepatic nodules (≥ 1 cm). All participants underwent same-day SZUS (experimental method) and SVUS (control method) for one representative nodule per participant. Images were interpreted by three readers (the operator and two independent readers). All malignancies were diagnosed histopathologically, while the benignity of other lesions was confirmed by follow-up stability or pathology. The primary endpoint was per-lesion diagnostic accuracy for HCC pooled across three readers using the conventional contrast-enhanced ultrasound diagnostic criteria, including arterial phase hyperenhancement followed by mild (assessed within 2 minutes after contrast injection) and late (≥ 60 seconds with a delay of 5 minutes) washout. The noninferiority delta was -10%p. Furthermore, different time delays were compared as washout criteria in SZUS, including delays of 2, 5, and > 10 minutes.

Results: A total of 105 lesions (HCCs [n = 61], non-HCC malignancies [n = 19], and benign [n = 25]) were evaluated. Using the 5-minutes washout criterion, per-lesion accuracy of SZUS pooled across the three readers (72.4%; 95% confidence interval [CI], 64.1%–79.3%) was noninferior to that of SVUS (71.4%; 95% CI, 63.1%–78.6%), meeting the statistical criterion for non-inferiority (difference of 0.95%p; 95% CI, -3.8%p–5.7%p). The arterial phase hyperenhancement combined with the 5-minutes washout criterion showed the same sensitivity as that of the > 10-minutes criterion (59.0% vs. 59.0%, $p = 0.989$), and the specificities were not significantly different (90.9% vs. 86.4%, $p = 0.072$).

Conclusion: SZUS was noninferior to SVUS for diagnosing HCC in at-risk patients using the same diagnostic criteria. No significant improvement in HCC diagnosis was observed by extending the washout time delay from 5 to 10 minutes.

Keywords: *Sonazoid-enhanced ultrasound; SonoVue-enhanced ultrasound; Hepatocellular carcinoma; Noninferiority test*

INTRODUCTION

Contrast-enhanced ultrasound (CEUS) is a valuable tool for characterizing hepatic lesions without renal toxicity or radiation hazards and can be used for the noninvasive diagnosis of hepatocellular carcinoma (HCC) in high-risk

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Corresponding author: Jeong Min Lee, MD, PhD, Department of Radiology, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea.
• E-mail: jmsh@snu.ac.kr

individuals [1-4]. Given their real-time imaging accessibility, CEUS may circumvent mistiming issues in the arterial phase of CT or MRI, with a higher sensitivity for revealing arterial hyperenhancement [5-7]. More importantly, US contrast agents help differentiate vascular pseudolesions from HCC [8-10]. The CEUS Liver Imaging Reporting and Data System (LI-RADS) [11] proposed diagnostic criteria for HCC, composed of arterial phase hyperenhancement (APHE) with mild and late (≥ 60 seconds) washout, and the criteria were used in several guidelines, including the European Association for the Study of the Liver (EASL) [12,13]. Furthermore, the EASL guideline adopted CEUS using SonoVue as a second-line diagnostic modality [10,13-15]. A recent prospective study demonstrated that CEUS using SonoVue might increase the frequency of HCC diagnosis without changing the specificity when used as a second-line diagnostic modality after gadoxetate-enhanced MRI, according to the EASL guidelines [16].

More recently, Sonazoid™ (Perfluorobutane; GE Healthcare), a Kupffer agent, has been available in a few countries, such as Japan, Korea, China, and Norway [17]. Sonazoid bubbles are taken up by the reticuloendothelial system (RES) and demonstrate a "Kupffer phase," which yields a sustained liver parenchymal enhancement for at least one hour [10,18-20]. Several studies have reported very high sensitivity of Sonazoid for detecting HCC using Kupffer phase imaging [21,22]. According to the Asian Pacific Association for the Study of the Liver and the Japan Society of Hepatology, Sonazoid-enhanced US (SZUS) is the recommended secondary diagnostic modality for indeterminate nodules on CT or MRI [23,24].

However, to date, only a limited number of studies have compared SonoVue-enhanced US (SVUS) with SZUS for HCC diagnosis [25-27]. A recent prospective study of high-risk participants suggested that SZUS provided higher sensitivity but similar specificity to SVUS using the same criteria [25]. However, it included only 59 participants from a single center, and many HCCs were diagnosed non-invasively; thus, its generalizability is relatively weak. The other two prospective studies demonstrated that SZUS showed noninferiority compared with SVUS for differentiation of benign and malignant lesions, using a noninferiority margin of 20%p [26,27]. However, it is unclear whether the diagnostic performance of SZUS for HCCs is inferior to that of SVUS.

Therefore, we aimed to determine whether SZUS was noninferior to SVUS for diagnosing HCC if the same diagnostic criteria were used for HCC diagnosis in at-risk participants and to suggest the most appropriate time delay to assess washout in the diagnosis of HCC using SZUS.

MATERIALS AND METHODS

Study Design

This prospective, single-center, noninferiority study (NCT04847726) recruited participants at risk of HCC who had treatment-naïve solid hepatic lesions (≥ 1 cm) at an academic tertiary care center in Korea between June 2020 and July 2021. The primary endpoint was the per-lesion diagnostic accuracy of SZUS and SVUS for HCC using the same diagnostic criteria, including non-rim APHE (≥ 1 cm) with mild and late (≥ 60 seconds) washouts [13,25]. A mild degree of washout was evaluated within 2 minutes after contrast injection, and late washout was assessed with a delay of 5 minutes. Additional study outcomes were per-lesion sensitivity and specificity of SZUS for diagnosing HCC using different time delay criteria for washout. We compared three time delays as washout criteria to investigate the impact of RES uptake on washout, including delays of 2, 5, and > 10 minutes (Kupffer phase).

Participants

This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board (IRB No. H-1807-166-962) of our institute. Written informed consent was obtained from all the participants. The inclusion criteria were as follows: 1) adult participants (≥ 18 years) at risk of HCC [13,28,29], 2) at least one treatment-naïve solid hepatic lesion (≥ 1 cm) on conventional US, CT, or MRI within four weeks of study enrollment, and 3) being scheduled for hepatic surgery or percutaneous biopsy for hepatic lesions, or hepatic lesions with more than two years of follow-up. The exclusion criteria were as follows: 1) definitely or probably benign non-tumorous hepatic lesions, such as intrahepatic portosystemic venous shunt, perfusion alteration, hepatic fat sparing or deposition, or confluent fibrosis [30], 2) expected insufficient diagnosis, not enough to ensure more than two years of stability or pathologic diagnosis, 3) apparent tumor in vein, 4) congestive hepatopathies, and 5) refusal to enroll in this study. When CT or MRI depicted multiple eligible lesions, one representative lesion per participant was analyzed based on predetermined criteria as follows: 1)

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Korean Journal of Radiology
KJR

an observation possessing a higher probability of hepatic malignancy according to CT/MRI LI-RADS version 2018, 2) being close to the skin, 3) better visibility on B-mode US, and 4) manageable tumor size (< 10 cm, considering the scan coverage of a convex US probe).

Contrast-Enhanced Ultrasound

Real-time CEUS was performed by one of the two board-certified abdominal radiologists (with 25 and 9 years of experience in abdominal US, and 12 and 5 years of experience in CEUS, respectively), who were level III experts according to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) [31], using a contrast-specific US platform (RS80A [n = 97] or RS85A [n = 7], Samsung Medison; LOGIQ E10 [n = 8], GE Healthcare) with a convex probe. Predetermined US parameters differed depending on the contrast agent and US platform. The mechanical index (MI) used for Sonazoid and SonoVue were 0.19–0.22 and 0.08–0.14, respectively. The detailed parameters are listed in the Supplementary Table 1. The operators were allowed to adjust the US parameters to optimally depict the lesions. For indistinguishable lesions on B-mode images, real-time US images were combined with CT/MRI scans for accurate examination (n = 10, 8.9%). The contrast agents, Sonazoid (Perfluorobutane, GE Healthcare) and SonoVue (Sulfur Hexafluoride, Bracco), were prepared according to the manufacturer's recommendations and manually injected via a venous cannula followed by flushing with 10 mL of normal saline. The timer was started at the beginning of the saline flushing. After performing SVUS, B-mode scanning with a high MI impulse was performed to ensure microbubble degradation. SZUS was performed at least 30 minutes later. Continuous CEUS clips of the target lesion were recorded during normal calm breathing for the first 70 seconds and then intermittently scanned every 20 seconds for 5 minutes after SonoVue injection or 10 minutes after Sonazoid injection.

Image Analysis

The operators recorded the following dynamic features on the structured report form: the presence of APHE and its pattern (rim, non-rim, and peripheral globular), washout timing, and degree (mild and marked) in both SVUS and SZUS. Echogenicity in the Kupffer phase was also recorded in SZUS. The Kupffer phase was defined as the phase 10 minutes after Sonazoid injection [32]. The dynamic features are defined in the Supplement. Two additional independent reviewers, who were board-certified abdominal radiologists (with 14 and 8 years of experience in abdominal US, and 6 and 4 years of experience in CEUS, respectively), and level III experts according to the EFSUMB [31], independently reviewed the stored images and recorded the aforementioned dynamic image features of SVUS and SZUS with at least two weeks review interval. The reviewers were blinded to the final diagnosis, any clinical or laboratory information, and the results of prior contrast-enhanced MRI or CT; however, they were aware that the study population was at risk of HCC and were given the size and location of each lesion.

Reference Standard

Eighty-two percent (86 of 105) of the target lesions were diagnosed histopathologically (surgery, n = 57; biopsy, n = 30) and 17% (18 of 105) were diagnosed noninvasively. Information on hepatic tumor pathology and immunohistochemistry was routinely described in our institution's pathology reports by one of the two experienced pathologists with more than 19 years of experience in hepatic pathology. All malignancies and some benign lesions with available data were diagnosed by pathology. For lesions without pathological confirmation, benignity was confirmed based on their stability for more than two years. Specific diagnoses were made if the lesions showed the typical imaging features of hemangiomas on CT or MRI. Otherwise, the lesions were considered nonspecific benign lesions. Images taken before study enrollment were used to ensure long-term stability of the lesion.

Statistical Analysis

The noninferiority of SZUS compared with SVUS regarding the primary endpoint, i.e., per-lesion diagnostic accuracy for HCC, was tested by comparing the lower margin of the two-sided 95% CI of their difference (SZUS - SVUS) with a noninferiority margin of -10%p [25]. The power of the study was approximately 98% at a one-sided significance level of 2.5% in the McNemar test, when the accuracies of SZUS and SVUS were assumed to be 90% and 80%, respectively [25]. The proportion of disagreement, a nuisance parameter for calculating sample size, was assumed to be 26%. The required number of subjects for the primary analysis was 112, assuming a 5% dropout rate by using PASS statistical software version 20.0.3 (NCSS). The 95% CI for the difference between two correlated accuracies obtained by each reviewer was estimated using a method

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based on the Wilson score interval [33]. The sensitivity, specificity, and accuracy with their 95% CI for the pooled data across the operator and reviewers 1 and 2 were estimated using generalized estimating equation using logit link with an exchangeable working correlation structure to account for the correlation among three interpretations per examination. For the primary analysis, the 95% CI for the difference in estimated accuracy pooled across the three readers (operator and reviewers 1 and 2) was used [34]. Additionally, interobserver agreement between the reviewers and operator was estimated using Gwet's agreement coefficient.

If noninferiority was confirmed, the per-lesion sensitivity and specificity of SZUS for diagnosing HCC using different time delays as washout criteria were compared using a generalized estimating equation. The time delays for washout included delays of 2, 5, and > 10 minutes (Kupffer phase) after contrast injection. When the overall *p* value was statistically significant ($p < 0.05$), pairwise differences were tested using the Hochberg method to control the inflation of the type I error for multiple testing.

Statistical analyses were performed using SAS version 9.4 (SAS Institute) and MedCalc version 16.4 (MedCalc Software).

RESULTS

A total of 160 participants were screened from June 2020 to July 2021; 112 were eligible for this study and scheduled for CEUS. Seven participants with invisible lesions, even after real-time US fusion with CT or MRI, were excluded

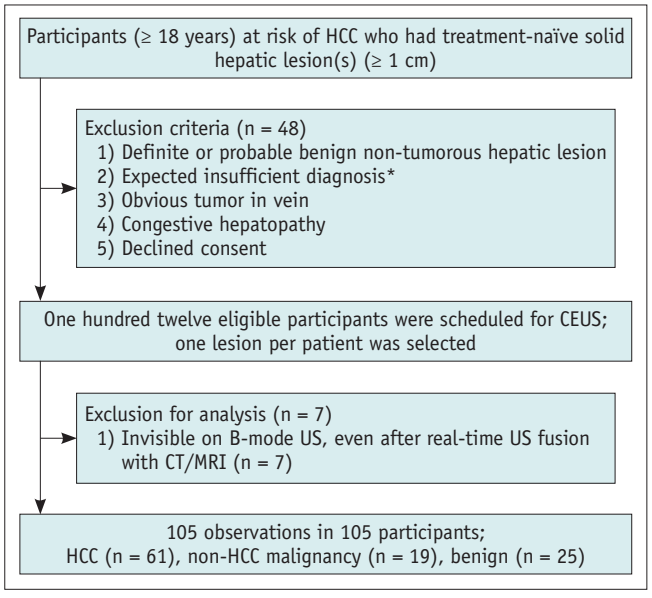

Fig. 1. Flow diagram of study. *Not enough to ensure more than two-year stability nor pathological diagnosis. CE = contrast-enhanced, HCC = hepatocellular carcinoma, US = ultrasound

Table 1. Clinicopathological Characteristics of 105 Participants with 105 Focal Hepatic Lesions

Participants	n = 105
Sex	
Male:female	71:34
Age, years	63 ± 11
Cause of liver disease	
Hepatitis B virus	74 (70.5)
Hepatitis C virus	3 (2.8)
Alcohol	9 (8.6)
NAFLD	11 (10.5)
Others	8 (7.6)
Known cirrhosis	55 (52.4)
Child–Pugh classification	
Score 5	96 (91.4)
Score 6	9 (8.6)
AFP level, ng/mL	587.3 (1.2–32770)
PIVKA-II, mAU/mL	649.6 (12–10425)
Hepatic Lesions	**n = 105**
Size, mm	33.1 ± 21
10–50	85 (81)
> 50	20 (19)
Final diagnosis	
HCC	61 (58.1)
Non-HCC malignancy	
cHCC-CC	7 (6.7)
IHCC	9 (8.6)
Metastasis	3 (2.8)
Benign	
Dysplastic nodule	9 (8.6)
Hemangioma	9 (8.6)
AML	3 (2.8)
Hepatic adenoma	2 (1.9)
Inflammatory lesion	2 (1.9)
Standard reference of diagnosis	
Operation	57 (54.3)
Biopsy	30 (28.6)
Presumed benign*	18 (17.1)

Data are mean ± standard deviation or median (range) for continuous variables and number of patients or lesions with % in parentheses for others. *Presumed to be benign without specific diagnosis based on their stability for more than two years (n = 9) or typical imaging features of hemangioma (n = 9) with more than six months of stability. AFP = alpha-fetoprotein, AML = angiomyolipoma, cHCC-CC = combined hepatocellular carcinoma and cholangiocarcinoma, HCC = hepatocellular carcinoma, IHCC = intrahepatic cholangiocarcinoma, NAFLD = nonalcoholic fatty liver disease, PIVKA-II = protein induced by vitamin K absence or antagonist II

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Korean Journal of Radiology
KJR

from analysis. Accordingly, 105 participants (71 male; mean age, 63 ± 11 years; range, 26–86 years) with 105 lesions (mean size, 33.1 ± 21 mm; range, 10–108 mm) were finally included (Fig. 1). Of these lesions, 58.1% (61 of 105) were HCCs, 18.1% (19 of 105) were non-HCC malignancies, and 23.8% (25 of 105) were benign lesions. The most common etiology of liver disease was hepatitis B virus infection (70.5% [74 of 105]). Fifty-two percent (55 of 105) of the participants had liver cirrhosis. All participants were Child-Pugh Class A. The baseline characteristics of the participants and target lesions are presented in Table 1.

Comparison between Diagnostic Accuracy of SZUS and SVUS

The per-lesion diagnostic accuracy pooled across by the three readers was 72.4% (95% CI, 67.1%–77.3%) for SZUS and 71.4% (95% CI, 66.1%–76.4%) for SVUS (Table 2, Fig. 2). The difference between SZUS and SVUS, which was 0.95%p (95% CI, -3.8%p–5.7%p), was above the -10%p noninferiority margin. However, superiority of the per-lesion diagnostic accuracy was not achieved. Two false-positive cases of SZUS, a hemangioma (Fig. 3) and a hepatic adenoma, presented true negative results on SVUS. Angiomyolipoma (AML) had a false-positive result on both

Table 2. Per-Lesion Diagnostic Accuracy, and the Number of TP, TN, FP and FN Lesions in SZUS and SVUS

	Operator	Reviewer 1	Reviewer 2	Pooled Data
SVUS				
Accuracy, %	71.4 (61.8, 79.8)	69.5 (59.8, 78.1)	73.3 (63.8, 81.5)	71.4 (66.1, 76.4)
TP, TN, FP, FN*	32, 43, 1, 29	34, 39, 5, 27	36, 41, 3, 25	
SZUS				
Accuracy, %	73.3 (63.8, 81.5)	71.4 (61.8, 79.8)	72.4 (62.8, 80.7)	72.4 (67.1, 77.3)
TP, TN, FP, FN*	36, 41, 3, 25	36, 39, 5, 25	36, 40, 4, 25	
Difference, %p (SZUS - SVUS)	1.9 (-6.8, 10.7)	1.9 (-6.0, 9.8)	-0.95 (-9.1, 7.2)	0.95 (-3.8, 5.7)

Unless otherwise noted, data are percentage with 95% confidence interval in parentheses. *Data are the number of lesions. FN = false negative, FP = false positive, SVUS = SonoVue-enhanced ultrasound, SZUS = Sonazoid-enhanced ultrasound, TN = true negative, TP = true positive

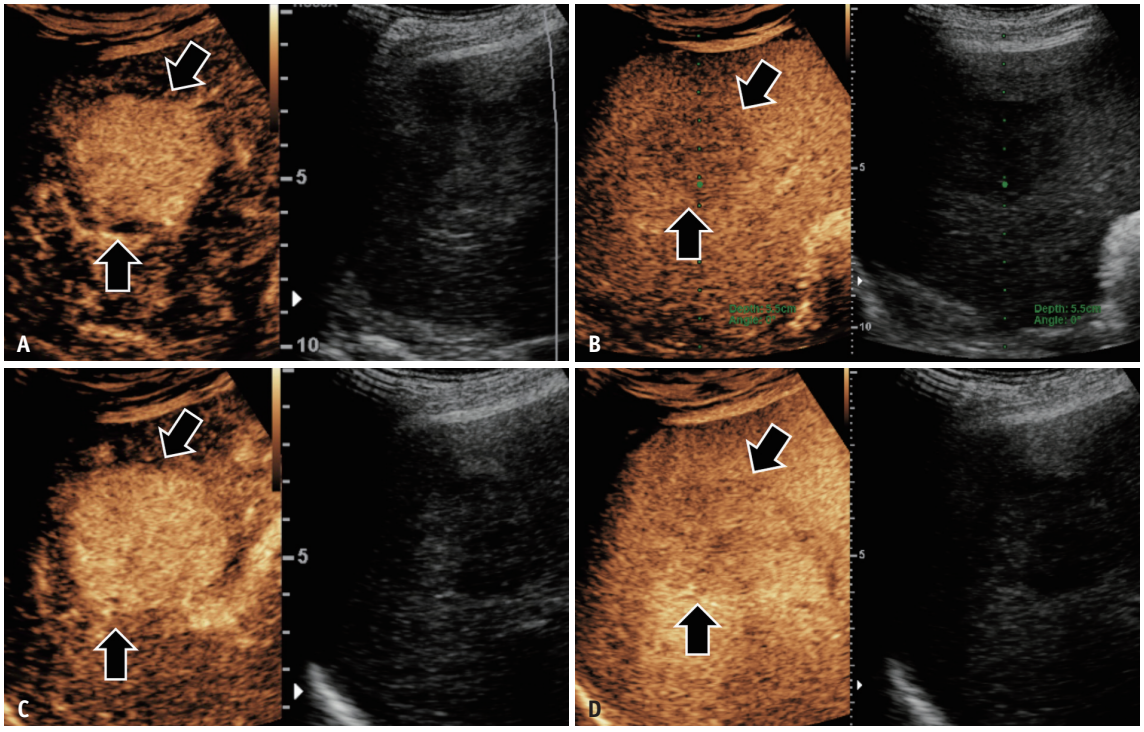

Fig. 2. A 70-year-old male with pathologically confirmed hepatocellular carcinoma in segment 6 of the liver.
A, B. On SonoVue-enhanced ultrasound, a 4.1 cm APHE (**A,** arrows) in segment 6 presented mild washout 155 seconds after contrast agent injection (**B,** arrows). **C, D.** On SZUS, a 4.1 cm APHE (**C,** arrows) showed mild washout 133 seconds after contrast agent injection (**D,** arrows). APHE = arterial phase hyperenhancement

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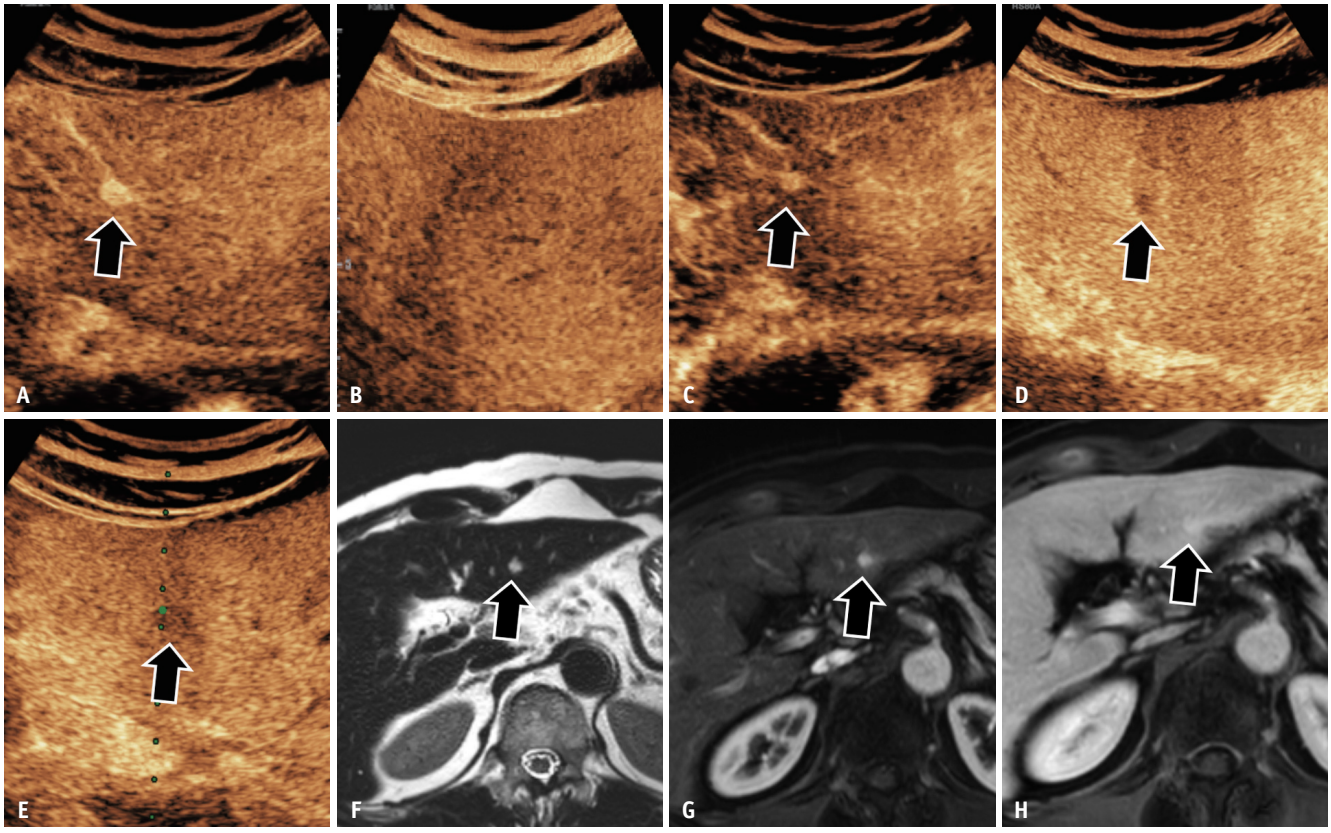

Fig. 3. A 62-year-old male with hemangioma in segment 3 of liver.
A, B. On SonoVue-enhanced ultrasound, a 1 cm APHE (**A,** arrow) in segment 3 did not present washout until 5 minutes after contrast agent injection **(B)**. **C-E.** On Sonazoid-enhanced ultrasound, a 1 cm APHE (**C,** arrow) showed mild washout 157 seconds after contrast injection (**D,** arrow), and presented low echogenicity on the Kupffer phase (**E,** arrow). **F-H.** This lesion was a hemangioma and showed bright signal intensity on T2-weighted images (**F,** arrow), and hyperenhancement in (**G,** arrow) arterial and (**H,** arrow) portal venous phases. APHE = arterial phase hyperenhancement

SZUS and SVUS. There were 25 false-negative cases with SZUS, and eight (32.0%) were further diagnosed with HCC using SVUS. On the contrary, there were 29 false-negative cases with SVUS and 12 (41.4%) showed true-positive results on SZUS.

Comparison of Different Time Delays as Washout Criteria on SZUS

The diagnostic performance of SZUS using different washout time delays is presented in Table 3. Using the 2-minutes criterion, the per-lesion specificity (98.5%; 95% CI, 94.6%–99.8%) was marginally high (p = 0.072), whereas the sensitivity (24.6%; 95% CI, 18.5%–31.5%) was lowest (p < 0.001). The sensitivity was the same between the 5-minutes and 10-minutes criteria (59.0%; 95% CI, 51.5%–66.2%). The specificity was not significantly different between the 5-minutes and 10-minutes criteria (90.9% vs. 86.4%, p = 0.072). Two more false-positive cases were noted when using the 10-minutes criterion and not the 5-minutes criterion (Fig. 4). They were confirmed to be metastases from hepatoid adenocarcinoma of the stomach and intrahepatic cholangiocarcinoma. Extending the washout time window from 5 to 10 minutes did not improve the diagnosis of any HCC cases.

DISCUSSION

In this prospective, noninferiority clinical trial, we found that when conventional CEUS diagnostic criteria, including APHE followed by mild and late (≥ 60 seconds with a delay of 5 minutes) washout, were used for HCC diagnosis, SZUS showed noninferiority to SVUS in per-lesion diagnostic accuracy for HCC diagnosis. The per-lesion diagnostic accuracy values of SZUS and SVUS were 72.4% and 71.4%, respectively, and the difference in diagnostic accuracy was 0.95%. There were also no significant differences in the pooled sensitivity and specificity of either CEUS agent. Our study is the first clinical trial to

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Korean Journal of Radiology
KJR

show the noninferiority of SZUS to SVUS in diagnosing HCC via intraindividual comparison in high-risk populations. Our results agreed with recent phase 3 clinical trial results, which reported no difference in the efficacy of SZUS and SVUS in differentiating malignancies from benign lesions [26,27]. According to the diagnostic algorithms of the

Table 3. Per-Lesion Sensitivity and Specificity of SZUS Using Different Time Windows for Washout

	Diagnostic Criteria	Sensitivity	Specificity	Accuracy	Interobserver Agreement*
Operator	SVUS	52.5 (39.3, 65.4)	97.7 (87.9, 99.9)	71.4 (61.8, 79.8)	
	SZUS, washout until 2 min	22.9 (13.2, 35.5)	97.7 (87.9, 99.9)	54.3 (44.3, 64.0)	
	SZUS, washout until 5 min	59.0 (45.7, 71.5)	93.2 (81.3, 98.6)	73.3 (63.8, 81.5)	
	SZUS, washout until > 10 min	59.0 (45.7, 71.5)	90.9 (78.3, 97.5)	72.4 (62.8, 80.7)	
Reviewer 1	SVUS	55.7 (42.4, 68.5)	88.6 (75.4, 96.2)	69.5 (59.8, 78.1)	
	SZUS, washout until 2 min	29.5 (18.5, 42.6)	97.7 (87.9, 99.9)	58.1 (48.1, 67.7)	
	SZUS, washout until 5 min	59.0 (45.7, 71.5)	88.6 (75.4, 96.2)	71.4 (61.8, 79.8)	
	SZUS, washout until > 10 min	59.0 (45.7, 71.5)	81.8 (67.3, 91.8)	68.6 (58.8, 77.3)	
Reviewer 2	SVUS	59.1 (45.7, 71.5)	93.2 (81.3, 98.6)	73.3 (63.8, 81.5)	
	SZUS, washout until 2 min	21.3 (11.9, 33.7)	100.0 (91.9, 100)	54.3 (44.3, 64.0)	
	SZUS, washout until 5 min	59.1 (45.7, 71.5)	91.0 (78.3, 97.5)	72.4 (62.8, 80.7)	
	SZUS, washout until > 10 min	59.1 (45.7, 71.5)	86.4 (72.6, 94.8)	70.5 (60.8, 78.9)	
Pooled data	SVUS (1)	55.7 (48.2, 63.1)	93.2 (87.5, 96.8)	71.4 (66.1, 76.4)	0.79 (0.70, 0.88)
	SZUS, washout until 2 min (2)	24.6 (18.5, 31.5)	98.5 (94.6, 99.8)	55.6 (49.9, 61.1)	0.86 (0.80, 0.93)
	SZUS, washout until 5 min (3)	59.0 (51.5, 66.2)	90.9 (84.7, 95.2)	72.4 (67.1, 77.2)	0.77 (0.68, 0.87)
	SZUS, washout until > 10 min (4)	59.0 (51.5, 66.2)	86.4 (79.3, 91.7)	70.5 (65.1, 75.5)	0.74 (0.64, 0.84)
p values	Overall†	< 0.001	0.072	< 0.001	
	(1) vs. (3)‡	0.578	N/A	0.802	
	(2) vs. (3)‡	< 0.001	N/A	< 0.001	
	(2) vs. (4)‡	< 0.001	N/A	0.002	
	(3) vs. (4)‡	N/A	N/A	0.125	

Unless otherwise noted, data are percentage with 95% confidence interval in parentheses. *Data are Gwet's AC1 values among three readers, †Overall *p* value comparing diagnostic performance among the three methods, ‡Adjusted *p* value by the Hochberg method. SVUS = SonoVue-enhanced ultrasound, SZUS = Sonazoid-enhanced ultrasound

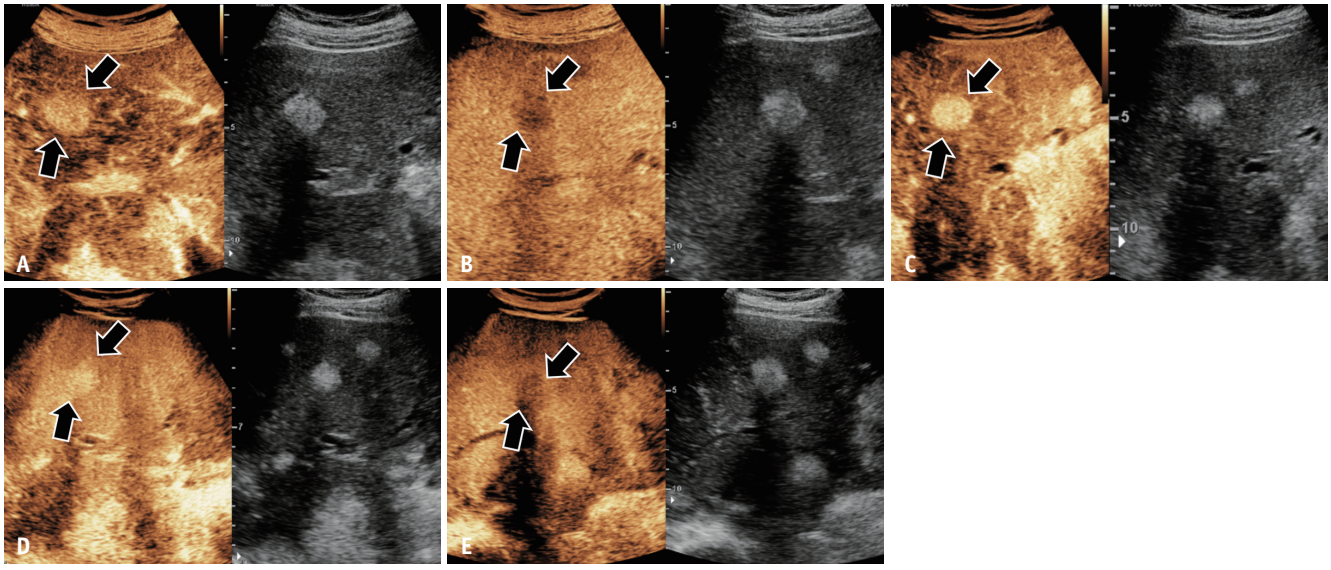

Fig. 4. A 60-year-old female with pathologically confirmed hepatoid adenocarcinoma from the stomach in segment 6 of the liver.
A, B. On SonoVue-enhanced ultrasound, a 1.9 cm APHE (**A,** arrows) in segment 6 presented mild washout 131 seconds after contrast agent injection (**B,** arrows). **C-E.** On Sonazoid-enhanced ultrasound, a 1.9 cm APHE (**C,** arrows) did not show washout until 5 minutes after contrast injection (**D,** arrows), and presented low echogenicity on the Kupffer phase (**E,** arrows). APHE = arterial phase hyperenhancement

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EASL and Korean Liver Cancer Association-National Cancer Center guidelines for HCC diagnosis, SVUS can be used as a secondary diagnostic modality after CT or MRI [13,28]. Based on our results showing the noninferiority of SZUS to SVUS, we suggest that SZUS can be used as a diagnostic tool for noninvasive diagnosis of HCC, similar to SVUS in patients at risk for HCC.

In addition, when the conventional CEUS diagnostic criteria for Sonazoid and SonoVue was used, both showed similar specificity values (90.9% vs. 93.2%). According to pharmacokinetic studies, Sonazoid is sequestered by RES via phagocytosis or adherence to the RES cells [19,20]. It helps identify target lesions using long-standing parenchymal enhancement (Kupffer phase) in percutaneous procedures, such as biopsy or radiofrequency ablation. However, for the diagnosis of HCC, the exact starting time of phagocytosis or adherence of Sonazoid bubbles to Kupffer cells, which may affect the timing or degree of washout, is still unknown [23,28]. Theoretically, more substantial hepatic parenchymal enhancement during the transitional (vasculo-Kupffer) phase may affect the degree or frequency of washout of focal liver lesions, as shown in previous studies using gadoxetate-enhanced MRI [35-37]. To date, there is a lack of consensus regarding the washout timing of SZUS, although the Kupffer phase is widely accepted, that is, 10 minutes post-injection [18-20,38]. In our study, when the diagnostic performance of three "washout" criteria with different washout timings was compared, the 2-minutes criterion yielded the highest per-lesion specificity (98.5%) and lowest sensitivity (24.6%). In addition, although the 5-minutes and 10-minutes criteria showed the same sensitivity value (59%) and similar specificity values (90.9% vs. 86.4%), two more false-positive cases (metastasis from hepatoid adenocarcinoma of the stomach and intrahepatic cholangiocarcinoma) were noted using the 10-minutes criterion but not the 5-minutes criterion. The good liver function of our participants might have led to the rapid uptake of Sonazoid in the RES system [18], which may be the reason for the lack of a significant difference in the sensitivity between the 5-minutes and 10-minutes criteria. Our results agree with those of previous studies [38], showing that the addition of Kupffer phase imaging did not improve the sensitivity for HCC diagnosis. However, several retrospective studies [21,22,39] reported better sensitivity for HCC diagnosis without compromising specificity by the addition of the Kupffer phase, although there were problems with weak reference standards and limited inclusion of various focal liver lesions such as cholangiocarcinoma, combined hepatocellular and cholangiocarcinoma, or hemangiomas. Thus, it remains uncertain whether adding the Kupffer phase to vascular or vasculo-Kupffer phase imaging can improve diagnostic accuracy without decreasing specificity, particularly in patients with decreased liver function. Further large-population studies, ideally multinational and multicenter studies, are necessary to define the additional benefits of Kupffer phase imaging.

When we used conventional CEUS diagnostic criteria for SZUS and SVUS, both showed similar sensitivity values (59.0% vs. 55.7%). Our results are similar to those of comparative studies between SZUS and SVUS for focal liver lesion characterization, which reported slightly inferior results with Sonazoid, although statistically insignificant [26,27]. However, our results differed from those of a previous prospective study [25] that reported a higher sensitivity of SZUS than SVUS. The reason for this discrepancy is not clear; however, it could be explained by the differences in reference standards: a higher proportion of noninvasive diagnoses in the previous study than in our study (37.4% vs. 17.1%). For example, in our study, three AMLs showed APHE and washout in the portal venous phase on gadoxetate-enhanced MRI. If these AMLs were diagnosed noninvasively, they may have been diagnosed with HCC as a reference. One of them presented with the LR-5 feature in SZUS, resulting in decreased specificity. Considering that our study population included many hepatitis B-related chronic hepatitis or cirrhosis cases, our results need to be confirmed in a larger study population with various etiologies of liver cirrhosis.

In our study, we reported < 60% sensitivity in both SZUS and SVUS. The relatively low sensitivity and high specificity of CEUS are consistently reported in previous studies [1,40]. The strict washout criteria for HCC diagnosis may explain the low sensitivity of CEUS. Earlier studies [41,42] revealed that the degree and time of washout are important in differentiating cholangiocarcinoma from HCC, and this criterion is widely accepted to maintain substantial specificity. In a previous study which determined late washout of CEUS in HCC diagnosis [38], when adopting the earlier cutoff for late washout from 60 seconds to 50 seconds, the sensitivity increased, but one of the 31 non-HCC lesions was diagnosed as HCC.

This study had some limitations. First, our clinical trial was performed in a single tertiary center in a region

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Korean Journal of Radiology
KJR

with a very high prevalence of hepatitis B infection and related cirrhosis. Therefore, the extent of our trial results may be limited for application to Western populations. However, as our study was performed using a noninferiority study design with an intra-individual comparison of two contrast agents, we believe that the intended purpose was adequately addressed in the study. Second, the number of pathologically confirmed benign cases was small. Nonetheless, we used a sufficiently long follow-up period to establish the stability of these lesions, as well as the typical imaging features of other dynamic imaging studies. Given the nature of the clinical practice of infrequently performing biopsies for benign liver lesions, this is an unavoidable limitation of CEUS studies. Finally, SVUS was performed earlier than SZUS because the half-life of Sonazoid is approximately 40 minutes and lasts several hours. Therefore, it is possible that the diagnostic performance of SZUS was slightly overestimated. However, two reviewers performed an additional blinded review of the CEUS examinations in random order; therefore, this might not be a serious problem.

In conclusion, SZUS was noninferior to SVUS for diagnosing HCC in at-risk patients. Furthermore, because the lesions met the LR-5 criteria within 5-minutes after contrast injection with SZUS, Kupffer imaging may not be mandatory for diagnosing HCC.

Supplement

The Supplement is available with this article at https://doi.org/10.3348/kjr.2022.0388.

Availability of Data and Material

Data generated or analyzed during the study are available from the corresponding author by request.

Conflicts of Interest

Jeong Min Lee and Ijin Joo who is on the editorial board of the *Korean Journal of Radiology* was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

Author Contributions

Conceptualization: Jeong Min Lee, Hyo-Jin Kang. Data curation: Jeong Min Lee, Hyo-Jin Kang. Formal analysis: Hyo-Jin Kang, Jeong Hee Yoon, Jeongin Yoo, Yunhee Choi. Funding acquisition: Jeong Min Lee. Investigation: Jeong Min Lee, Hyo-Jin Kang. Methodology: Jeong Min Lee, Hyo-Jin Kang, Yunhee Choi, Ijin Joo. Project administration: Jeong Min Lee. Resources: Jeong Min Lee, Joon Koo Han. Software: Jeong Min Lee. Supervision: Jeong Min Lee, Yunhee Choi, Joon Koo Han. Validation: Hyo-Jin Kang, Jeong Hee Yoon, Jeongin Yoo, Ijin Joo. Visualization: Jeong Min Lee, Hyo-Jin Kang, Ijin Joo. Writing—original draft: Hyo-Jin Kang. Writing—review & editing: Jeong Min Lee, Jeong Hee Yoon, Ijin Joo.

ORCID iDs

Hyo-Jin Kang
https://orcid.org/0000-0002-6771-2112
Jeong Min Lee
https://orcid.org/0000-0003-0561-8777
Jeong Hee Yoon
https://orcid.org/0000-0002-9925-9973
Jeongin Yoo
https://orcid.org/0000-0002-3267-2544
Yunhee Choi
https://orcid.org/0000-0001-5305-1803
Ijin Joo
https://orcid.org/0000-0002-1341-4072
Joon Koo Han
https://orcid.org/0000-0001-5916-5545

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