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Sonazoid-enhanced intraoperative ultrasonography in patients with gall bladder diseases: A preliminary study

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Background. Ultrasonography using Sonazoid, a novel contrast agent containing microbubbles, is a useful diagnostic modality for intrahepatic mass lesions with specific characteristics in two phases: contrast enhancement of early vascular perfusion and perfusion defect of the lesion in the late phase. To improve the diagnostic accuracy of intraoperative ultrasonography (IOUS) in biliary neoplasms, particularly gall bladder, we investigated the usefulness of the Sonazoid contrast agent in a preliminary study.

Subjects and Methods. We examined IOUS images of 23 patients with gall bladder disease. Sonazoid was administered intravenously, and early arterial and venous phase images of the tumor were obtained during laparotomy.

Results. Tumors included the localized type of adenomyomatosis in the fundus of the gallbladder (GAM) in seven patients as benign controls, adenomatous diseases in three, and gallbladder carcinoma (GC) in 13. Sonazoid IOUS scanning was performed in all patients, with no adverse effects. Although GC showed high enhancement in 77% of the tumors, GAM and adenoma also showed mild enhancement in more than half of them. Pseudo-positivity was observed in one case of hyperplastic polyps, and pseudo-negativity was observed in two patients. Cholesterol polyps and a suspicious lesion of liver metastasis by the computed tomography were not enhanced with Sonazoid treatment.

Conclusion. Although there remains a problem of pseud-positivity in the intracystic lesions, Sonazoid IOUS may be a useful tool to detect vascularity and its location or extension of gallbladder diseases; however, neither significance of differential diagnosis with benign diseases nor other clinical significance was found.

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Introduction

The development of various preoperative techniques for imaging biliopancreatic neoplastic lesions has been remarkable,[1] but the diagnostic accuracy of tumor existence or extent is more precise when using intraoperative ultrasonography (IOUS).[2,3] IOUS is an essential tool to precisely determine tumor location and surgical margins. However, these are sometimes difficult to determine using only conventional IOUS.[4]

Contrast media-enhanced US was developed in the 1990s, and Levovist™ was used to examine the morphology of vascular flow in tumors in the field of liver malignancy.[5] In western and eastern Asian countries, the microbubble of absorbable agents as a contrast medium for vascular blood flow has been subsequently developed and widely applied to

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detect or evaluate hepato-biliopancreatic neoplastic lesions as well as organ perfusions,[6-8] However, many of these agents, including sulfur hexafluoride microbubbles (SonoVue®: Bracco, Milan, Italy), which is a medium with a stable biomaterial shell and has been widely used in the western series, cannot be applied in Japan because of the lack of insurance approval of these agents and related ultrasonography apparatus so far. Since the development of an advanced perfluorobutane microbubble agent named Sonazoid® (Daiichi Pharmaceutical, Tokyo, Japan, and GE Healthcare, Giles, UK) in recent years, the detection of liver malignancies with or without intratumoral vascularity has dramatically improved in Japan.[9, 10] This agent is more concentrated than the previously used agents and remains in the liver parenchyma for a long time after injection because of the superior stability, feasibility, and safety of these microbubbles and covered biomaterial shells.[9, 10] Sonazoid is usually applied to detect two phases of early phase vascular flow and perfusion pooling in normal organs and mass lesions, including malignancies. In the field of liver diagnosis, reperfusion techniques to determine intra-tumor perfusion during the arterial phase or negative perfusion imaging in the Kupffer phase (almost the same period as the late venous or parenchymal perfusion phase) in the liver can be conducted,[11] and metastatic liver lesions can also be easily detected.[12] The usefulness of Sonazoid IOUS for intraoperative diagnosis has been reported and validated, and comparisons with conventional non-contrast IOUS have been clarified, including in our studies.[13-16] Although the use of Sonazoid in the diagnosis of biliopancreatic neoplasms has varied,[17-20] the clinical usefulness of this type of IOUS has not yet been fully clarified. However, Sonazoid is very useful for detecting both hypervascular and hypovascular lesions in any organ, based on the specific characteristics of this agent at this stage. Therefore, we hypothesized that Sonazoid IOUS would be a useful tool for the differential diagnosis of malignancies and evaluation of the tumor extent in the primary lesion or liver metastases during laparotomy. Accurate diagnosis during surgery is important to achieve adequate curability even in gallbladder diseases during operation.

Thus, the present preliminary study aimed to clarify various aspects of Sonazoid IOUS use, and for this purpose, we assessed Sonazoid IOUS in 23 patients with gallbladder tumorous diseases in whom the differential diagnosis of the tumor could not be determined during laparotomy, in case decision of the intraoperative strategy was required and examined the feasibility or limitations of its use. The novel results of increased sensitivity or specificity of intracystic malignancy by the IOUS using Sonazoid would also be hypothesized and expected. The subjects were retrospectively and consecutively selected between 2010 and 2020 from two academic institutions where the first author helped to treat these subjects.

Patients and methods

The study protocol was reviewed and approved by the Clinical Research Support Center, University of Miyazaki Faculty of Medicine (approval number: O - 0890, February 9, 2021), and the Hospital Clinical Research Ethics Committee of Nagasaki University School of Biomedical Sciences (approval number:21031522, March 16, 2021). Informed consent for data collection was obtained by the opt-out procedures at the website for one month after permission, but no disclaimer was observed. Anesthesia and patient data were retrieved from both institutional databases.

The study subjects were 23 patients with liver tumors who were scheduled for surgery and admitted to the Division of Surgical Oncology at Nagasaki University School of Biomedical Sciences between January 2010 and March 2015, or the Division of Hepato-biliary-pancreatic Surgery, Department of Surgery, University of Miyazaki Faculty of Medicine between April 2015 and August 2020. These subjects using ultrasonographic diagnosis using Sonazoid was limited in cases where the decision of the intraoperative strategy for deciding operative extension was required by using any modality, including differential diagnosis by Sonazoid-US at the final step of the operation, but not examined in all surgeries for biliopancreatic diseases in our series. In this study, all patients were examined using Sonazoid IOUS after approval in 2007 by the Japanese health authorities. Gallbladder diseases included localized adenomyomatosis in the fundus of the gallbladder (GAM) in seven patients as benign controls as intracystic adenomatous diseases in three patients and gallbladder carcinoma (GC) in 13. The patients included 9 males and 14 females with a mean age at the time of surgery of 67.3 ± 9.2 years (±SD, range, 31−83 years). The background liver disease included a normal liver in 21 patients, fatty liver in one, and chronic viral hepatitis in one. The operative procedures included cholecystectomy in 10 patients and gallbladder bed resection in 13. Radical surgical resection was performed without macroscopic exposure of the amputated section to the remaining tissue.

Examination with IOUS and contrast-enhanced IOUS was performed using a Xario™ XG system (Toshiba Medical Systems, Tokyo, Japan) with a 3.5-MHz microconvex probe (PVT-375BT; Toshiba) and a 7.5-MHz inter-operative probe (PLT-705BTH, Toshiba) in the operating room of the Nagasaki

University Hospital until March 2015. In April 2015, an ALPHA7 system (Aloka, Tokyo, Japan) with a 1-MHz convex probe (UST-9146T, Aloka) and, subsequently, an ARIETTA E70 system (Aloka) with a 3.5-MHz convex probe (C42T, Aloka) were used in the operating room of Miyazaki University Hospital. All intraoperative IOUS examinations were performed by surgeons to determine tumor location and extent. We first examined two-dimensional (2D) images of the tumor and its location, as well as color Doppler images of the tumor vasculature.[21] We separately scanned the shallow area and the deep or bottom area of the region of interest by changing the intensity of the echo according to tumor depth. For tumors located on the organ surface, the surface was covered with warm saline to reduce the air gap between the probe and the liver surface. An intravenous bolus injection of 0.5 mL of perflubutane microbubbles (Sonazoid; Figure 1a-b) was administered via a peripheral vein at the time of laparotomy incision to observe the early arterial phase at 30–60 s and the late parenchymal perfusion phase image at approximately 20-30 minutes after injection because the microbubbles remained for a long time.[19] Contrast harmonic imaging of the liver and tumor was observed on the micro-flow images. Real-time views of the conventional 2D image and micro-flow images of Sonazoid IOUS can be observed on dual monitors. The late Kupffer phase image was observed under a low mechanical index (an index of the mechanical impact of the ultrasonic waves on the target) of 0.3, and scanning of the tumor lesion was performed to ensure the integrity of the Sonazoid microbubble structure because Sonazoid microbubbles can burst at a high mechanical index of over 0.3.[22] The primary tumor with high vascularity was expected to be identified as a high perfusion lesion contrasting with neighboring tissues (Figure 1c), and the hypovascular region was expected to appear as a low-contrast perfusion defect as debris (Figure 1d).

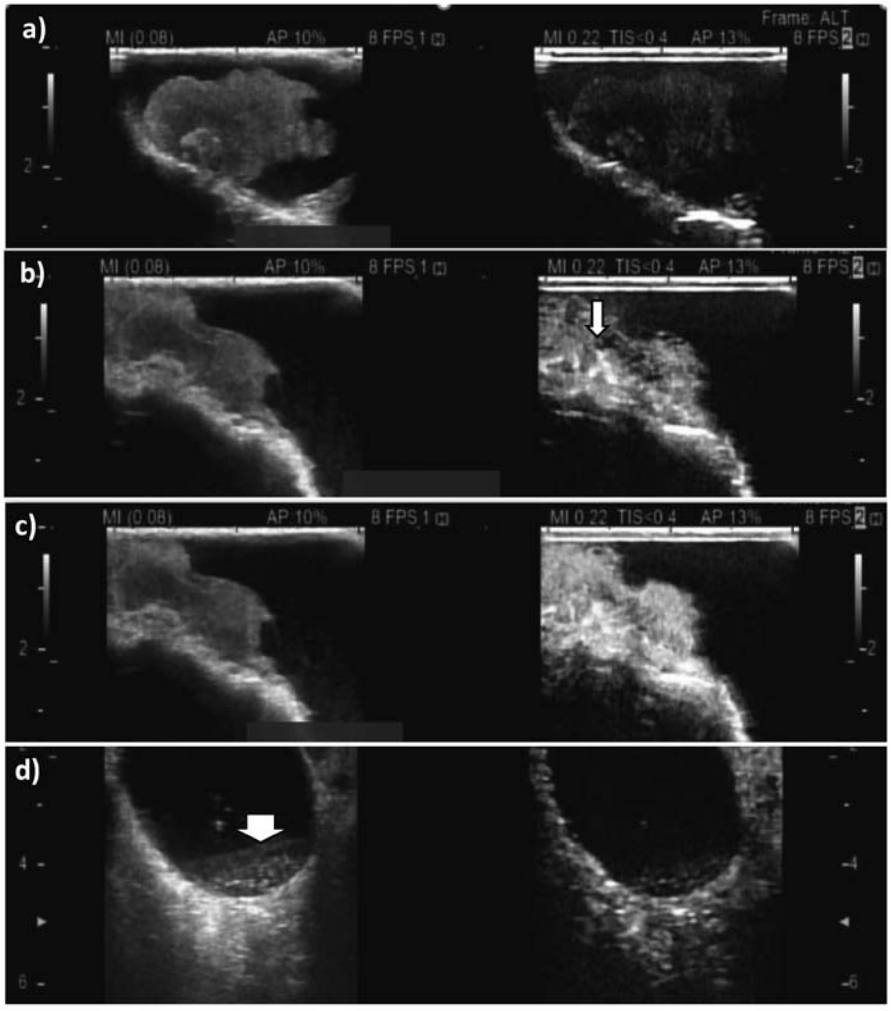

Figure 1. (a) The US image before administration of microbubble agent, Sonazoid. (b) Representative early arterial phase image of gallbladder carcinoma with arterial hypervascularity (arrow). (c) Intra-tumorous staining at the venous phase. d) Intra-cystic debris (thick arrow) was not enhanced.

Using this imaging technique, we examined the tumor morphology, surrounding tumor extension, vascular infiltration, scanning of other tumor lesions, and expected width of the surgical margin.

Results

Diagnosis of gallbladder tumors by Sonazoid IOUS

Sonazoid IOUS scanning was performed in all patients. Sonazoid injection was performed once in 21 of the 23 patients and twice in two patients. No adverse effects of the Sonazoid injection were observed in the present study. Vascularization of intra- and extra-tumorous lesions in the arterial or early portal phase was observed within 30 s in all patients. Table 1 shows the imaging findings of Sonazoid IOUS. Malignant gallbladder disease was present in 13 of the 23 patients (57%), adenomatous disease in three (13%), and benign inflammatory diseases in seven patients (29%). The main targeted lesion was well enhanced and was observed in 96% of the patients. One patient with adenomyomatos had a severely fatty liver, and it was difficult to scan the gallbladder and liver. In the arterial phase of Sonazoid enhancement, GC showed the highest enhancement compared with other benign lesions. However, all gallbladder adenomas and GAM showed good enhancement. Pseudo-positivity was observed in one patient with an accessory hyperplastic benign polyp (Figure 2a). Pseudo-negativity was observed in two patients: one with well-differentiated GC (Figure 2b). Cholesterol polyps and a suspicious lesion of liver metastases of well-enhanced adenocarcinomas were not enhanced by Sonazoid.

Table 1. Findings of gallbladder lesions by Sonazoid IOUS

1) Main tumors	
(1) Gall bladder and diseases	23
GAM	7
Low to moderate grade adenoma	3
Adenocarcinoma	13
(2) Detection of tumor lesion	
Well observed	22 (96%)
Not observed	1 (adenomyomatosis)
Sonazoid enhancement at the arterial phase	
Well stained	17/23(74%)
GAM	4/7 (57%)
Adenomatous lesion	3/3 (100%)
GC	10/13 (77%)
Not stained	6
2) Accessory lesions	
Well enhanced	1
Intra-cystic hyperplastic polyp	1
Not enhanced	3
Cholesterol polyp of gall bladder	1

GAM: Gallbladder adenomyomatosis of the fundus, GC: Gallbladder adenocarcinoma.

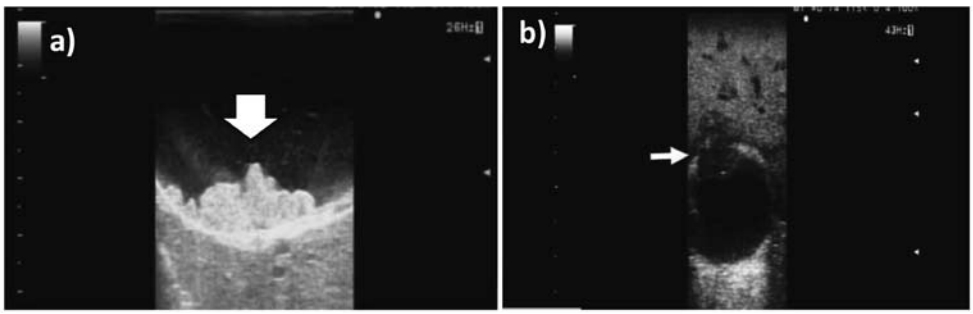

Figure 2. Pseudo-positivity of Sonazoid enhancement in accessory lesions. (a) hyperplastic benign polyp (thick arrow). Pseudo-negativity was observed in (b) gallbladder adenocarcinoma (thin arrow)

Surgical results using Sonazoid IOUS

All targeted lesions were resected, and a histopathological diagnosis was obtained from each specimen. All patients underwent complete or radical resection of both positive and negative lesions. In one patient with GC, the small, 8mm-in-size intrahepatic lesion at segment 8 suspected liver metastasis was pointed out preoperatively. However, intrahepatic mass lesions could not be detected even in both arterial and Kupffer phase enhancement. Thus, the scheduled gallbladder bed resection was performed. Otherwise, the operative extension was not changed during operation in this series.

Discussion

In 2007, Sonazoid, a new microbubble contrast media agent, was approved for use in Japan for the diagnosis of liver tumors; however, it has not been well applied in Western and other countries so far.[6-8, 16, 17, 23-25] In biliary and pancreatic diseases, Sonazoid is usually used to detect early phase vascular flow and perfusion pooling in normal organs, contrary to various malignant diseases as well as liver diseases. In this study, although we strongly expected the usefulness of Sonazoid-US for intraoperative diagnosis in deciding the final treatment strategy, it was very difficult to distinguish the differential diagnosis with highly enhanced benign lesions. No cases showed the change of operative extension as cholecystectomy or gallbladder bed resection. Therefore, this examination has not been routinely applied for all surgeries for biliary diseases, and there were not many selected subjects for 10 years in this series. The previous reports showed that diagnostic accuracy of vascularity or disease diagnosis by Sonazoid-US was not inferior to those by ultrasonography using Sulphur hexafluoride microbubbles (SonoVue) which is a similar agent with shell and has been well applied other worldwide countries.[8, 26-28] The introduction of this modality markedly improved the diagnostic accuracy of liver tumors to levels similar to those of gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid-magnetic resonance imaging (MRI).[13, 16, 24] The use of Sonazoid IOUS has allowed the detection of occult lesions or tumor spreading undetected by the plain US.[17, 25] Particularly, Kupffer-phase imaging is a powerful diagnostic tool to identify even small-sized abnormal nodules.[23, 24] The usefulness of IOUS has been well realized and is considered to be a reliable imaging technique often used to confirm the diagnosis of intrahepatic tumor lesions.[5, 9] In the present study, we expected that contrast IOUS using Sonazoid would enhance the detection of gallbladder lesions based on defining tumor vascularity and aid in the determination of sufficient surgical margin. The advantages of contrast IOUS using Sonazoid were recently reported in patients with liver tumors in our studies.[14, 15] However, to our knowledge, the significance of IOUS using Sonazoid has not been fully investigated in biliary neoplasms.[16-20] Therefore, its clinical usefulness has not been fully recognized. In the present preliminary study, we assessed the usefulness of Sonazoid IOUS in the detection of various gallbladder neoplasms and compared it with benign diseases. Sonazoid injection is extremely safe without severe side effects.[29]

Using the same Sonazoid IOUS protocol described in previous studies,[14, 15, 23] we first examined the early arterial phase, followed by vascularity in the portal phase during laparotomy in the present study. Sonazoid IOUS allows searching of the entire liver or pancreas over a relatively long period by screening for parenchymal perfusion. In the present series, all diseases were detected by either plain IOUS or Sonazoid IOUS. Among the limited number of 23 patients in this study, we attempted to determine non-GC lesions in 13 patients as a control; interestingly, GAM or adenoma as a control lesion showed an enhancement of over 50%; therefore, a significant difference in enhancement compared with that in GC was not observed. Although the higher speed or dense accumulation in Sonazoid was expected in GC compared to other benign diseases, these benign lesions may have a similar hypervascularity and malignancy. At this stage, this series could not define the significance of differential diagnosis between benign and malignancy. Other reports of contrast US findings in biliary diseases were reviewed between 2005 and 2020, and only a few investigated Sonazoid US for gallbladder carcinomas.[30, 31] Sugimoto et al. showed that contrast-enhanced endoscopic US enabled real-time observation of the hemodynamics of gallbladder tumors, which was applied for the diagnosis of malignancy.[30] Imazu et al. showed the usefulness of contrast-enhanced harmonic endoscopic US with Sonazoid in preoperative T-staging for pancreaticobiliary malignancies.[31] However, none of the reports showed an enhancement effect for conditions resembling cholecystitis or adenoma of the gallbladder. Our results showed that these conditions were also enhanced by Sonazoid IOUS due to tumor vascularization, although the positive rate tended to be lower, but not significantly different from that for gallbladder cancer. Sonazoid is difficult to use for differential diagnosis, even with sensitive IOUS. Although the result could not be well indicated in the present study, it is speculated that tumor location or extension, including infiltration, would be well detected both by hypervascularity in the early phase and wash-out or by hypoechoic lesions contrasting with the surrounding gallbladder wall and liver parenchyma in comparison with

the conventional US examination without (data not shown). A few investigators have reported vascularity in bile duct adenoma or hamartoma.[32-36] In our series, a cholesterol polyp was not enhanced due to less vascularity, as in the report by Yu et al.,[35] and, interestingly, debris formation resembling or adjacent to the main gallbladder cancer showed strong enhancement. Although we cannot certainly explain the enhancement of intracystic debris, the pseudo-positive or no-specific inflammatory content may disturb the differential diagnosis of this phenomenon even though the Sonazoid-use US. Hyperechoic artifacts were sometimes seen in sludge or debris due to the entrapment of cholesterol crystals, which may influence US detection. However, it is difficult to clearly explain this mechanism. The rare intrahepatic bile duct adenoma did not show enhancement on the Sonazoid US, contrary to the report by Wei et al. and findings in other adenomatous regions.[33] We speculated that the adenomatous origin was different from that of other adenomas.

Several reports have also shown the effectiveness of Sonazoid US in the intra-pancreatic region.[30, 31, 36] As a negative contrast imaging technique, this procedure may be able to potentially detect occult liver metastases or the minute invasive extent, which would be still suited to the field of biliary surgery. Thus, further studies in a larger series of patients with biliary disease including gallbladder will be necessary to clarify the echogenicity of disease vascularity.

Occult metastatic lesions, which are often newly detected by IOUS in approximately 20% of patients, require additional resection.[37, 38] In the present study, occult metastatic lesions were not detected using Sonazoid IOUS, which was different from the use of Sonazoid IOUS to detect occult metastatic lesions in intrahepatic malignancies. Compared with preoperative CT or MRI, it has been recently reported that conventional and Sonazoid IOUS is useful for detecting small accessory lesions during surgery.[14, 15] This information may be useful to avoid performing intraoperative surgical treatment that is too invasive. Based on the results of this preliminary study, the usefulness of Sonazoid IOUS was not clearly estimated; further studies with larger numbers of patients will be necessary in the future. The limitation of this study is that it was a preliminary retrospective cohort study with a small number of patients, which can be widely examined with a multi-institutional analysis. Drawbacks or shortcomings are supposed to be that the Sonazoid agent has not been well used worldwide nevertheless of ideal, accurate, and safe bubble agents in comparison to those used in other countries and commercial bases.

Conclusions

In this preliminary study, we showed that Sonazoid contrast-enhanced IOUS using the relatively novel perfluorobutane microbubble agent Sonazoid allowed easy examination of the location and extent of gallbladder tumors; however, the differential diagnosis between benign and malignant lesions with hypervascularity remains problematic. To assist in decision-making for surgical resection of this procedure, a prospective study should be necessary for the next step.

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Declarations

Funding

None

Conflict of interest / Competing interests

Atsushi Nanashima declares a conflict of interest with Shinnihon-Kagaku (SNBL) Ltd., Japan for the annual study fund.

Availability of data and material

The authors shared clinical data limited to the two academic institutions because the dataset is part of ongoing study protocols.

Code availability

Not applicable.

Authors' contributions

All the authors contributed substantially to this work. AN and YT conceived and designed the study. TH, MH, NI, MI, KY, KK, MI, YS, MN, and KH collected patient data. AN and YT analyzed and interpreted the data. AN initially drafted the manuscript and TN ensured the accuracy of the data and reconfirmed the study design. All authors have read and approved the final version of the manuscript and affirmed that the work has not been submitted or published elsewhere in whole or in part.

Ethics approval

All procedures involving human participants were performed

in accordance with the ethical standards of the institutional and/or national research committee and in line with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study was approved by the ethics review boards of both academic institutes described in the Methods section. The nature of the study was thoroughly explained to each patient, and informed consent was obtained by the opt-out method at institutional websites for a month before the study.

Consent for publication

Not applicable.

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