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ORIGINAL ARTICLE

Contrast-enhanced ultrasonography with Sonazoid for diagnosis of gangrenous cholecystitis

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Abstract
Purpose This prospective study investigated the ability of contrast-enhanced ultrasonography (CEUS) with Sonazoid to diagnose gangrenous cholecystitis and determined the inter-observer agreement.
Methods From September 2012 to August 2014, 27 patients with acute cholecystitis underwent preoperative CEUS (registration number 1277). After Sonazoid injection, harmonic imaging of the gallbladder wall was performed, and the findings were recorded using movie clips. The signal intensity was classified as absence (uncomplicated) or presence of perfusion defects (gangrenous). The physician performing CEUS recorded the findings immediately after the examination. Another physician (blinded to the clinical information) then reviewed the movie clips and recorded the findings. The final diagnosis was determined by histological examination in all 27 patients.
Results The final diagnosis was gangrenous cholecystitis in 15 patients and uncomplicated cholecystitis in 12. On CEUS examination, perfusion defects were detected in 10 patients with gangrenous cholecystitis, giving a sensitivity of 66.7 %, specificity of 100 %, positive predictive value of 100 %, and negative predictive value of 70.6 %. On review of the movie clips, these values were 73.3, 100, 100, and 75.0 %, respectively. The inter-observer agreement between physicians was good (κ coefficient, 0.64).
Conclusions CEUS with Sonazoid is a useful and reproducible modality for diagnosing gangrenous cholecystitis.

Keywords Contrast-enhanced ultrasonography · Gangrenous cholecystitis · Preoperative diagnosis

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Introduction

Evaluation of the severity of acute cholecystitis is essential for determining the optimal therapeutic strategy [1, 2]. Gangrenous cholecystitis should be carefully managed because it is associated with high morbidity and mortality; an urgent operation may be needed, and open rather than laparoscopic surgery is often required mainly because of the presence of severe adhesions [3, 4]. Therefore, preoperative differentiation between acute gangrenous and uncomplicated (non-gangrenous) cholecystitis is important. However, a preoperative diagnosis is not necessarily easy to achieve in daily practice.

Contrast-enhanced computed tomography (CECT) has good specificity (96.0–100.0 %) for the diagnosis of acute gangrenous cholecystitis; however, it has low sensitivity (29.3–70.6 %) [5–7], probably because of the difficulty in recognizing enhancement of the thin gallbladder wall. Although the most recent study of CECT [5] reported the

highest sensitivity (70.6 %) for diagnosing acute gangrenous cholecystitis, it included only a small number of subjects ($n = 25$). Additionally, some problems associated with CECT remain unresolved, such as the need for transportation to the radiology department and nephrotoxicity of the contrast agents.

Few reports have described the accuracy of magnetic resonance imaging for the diagnosis of acute gangrenous cholecystitis [8]. Therefore, the sensitivity and specificity of this modality remain unclear.

Although several reports have described the use of contrast-enhanced ultrasonography (CEUS) for the diagnosis of cholecystic diseases [9, 10], we found only one study focusing on the diagnosis of gangrenous cholecystitis by CEUS [11]; this study reported good diagnostic power using SonoVue. US has high spatial resolution; thus, we hypothesized that CEUS would be more useful than other imaging modalities for detecting the necrosis associated with gangrenous cholecystitis. In the present study, we investigated the accuracy of CEUS using the contrast agent Sonazoid (perflubutane; Daiichi Sankyo, Tokyo, Japan) for differentiating between gangrenous and uncomplicated cholecystitis in a prospectively enrolled series of patients with suspected acute cholecystitis. The overall aim of the study was to strengthen the evidence regarding the usefulness of CEUS. We also evaluated the inter-observer agreement between two physicians.

Methods

Patient selection

To estimate the number of cases before commencing this study, we referred retrospectively to the number of cases with acute cholecystitis at our hospital. According to our hospital data, we see up to 40 patients with acute cholecystitis, including 10 with gangrene, each year. Therefore, a study period of 2 years would be adequate to enroll the number of patients required for statistical analysis.

From September 2012 to August 2014, we investigated consecutive patients with suspected acute cholecystitis, who had clinical findings indicative of acute febrile illness with any abdominal symptoms and positive gray-scale US findings consistent with acute cholecystitis (short-axis gallbladder diameter >35 mm, gallbladder wall thickness >3.5 mm, and/or positive sonographic Murphy sign regardless of the presence of gallstones). Patients who provided informed consent underwent CEUS and were enrolled. Patients who did not undergo cholecystectomy and histological diagnosis after CEUS examination were excluded from this study.

The ethics committee of our institution approved the study protocol (Registration Number 1277).

US technique and interpretation

An US system (TUS-A500 or SSA-790A; Toshiba, Tokyo, Japan) equipped with 3–12-MHz transducers was used. Gray-scale US was performed for up to 10 min in each patient by one of five gastroenterologists with >10 years experience in abdominal US, and the US findings (gallbladder size, gallbladder wall thickness, irregularity of the mucosal surface, presence of gallbladder stones, sonographic Murphy sign, sonolucent layer, and presence of pericholecystic fluid) were recorded. If acute cholecystitis was suspected based on the US findings, a 0.015-mL/kg bolus of Sonazoid was injected into the left antecubital vein, followed by 10 mL of saline within 10 s. The gallbladder was then scanned using low-mechanical-index (0.2–0.3) harmonic imaging, and the images were recorded as a digital movie clip for 30 s, starting from recognition of the hepatic artery proper. During CEUS, the examiner scanned the entire segment of the gallbladder wall using adequate tilting of the probe and proper focusing of the US beam. The signal intensity obtained from the gallbladder wall during the arterial phase was classified as absence of perfusion defects (visual confirmation of signals from contrast agent in the mucosa of the whole gallbladder wall) (Fig. 1a, b) or presence of perfusion defects (signal defects of any size in the gallbladder wall) (Fig. 2a, b). Patients with absence of perfusion defects on CEUS were diagnosed with uncomplicated cholecystitis, and those with presence of perfusion defects were diagnosed with gangrenous cholecystitis. The CEUS findings were reported to the surgeons immediately after the examination, including whether the cholecystitis was judged to be gangrenous or uncomplicated. One of the other four physicians (blinded to the clinical information) subsequently reviewed the movie clips and recorded the diagnostic findings for investigation of the inter-observer variance.

Gold standard

We defined gangrenous cholecystitis as necrosis of any size in the wall of the gallbladder on macropathological and histological examination (Fig. 3), and uncomplicated cholecystitis as the absence of necrosis on macropathological and histological examination (Fig. 4).

Statistical analysis

The following characteristics were compared between patients with gangrenous cholecystitis (GC group) and patients with uncomplicated cholecystitis (UC group): sex,

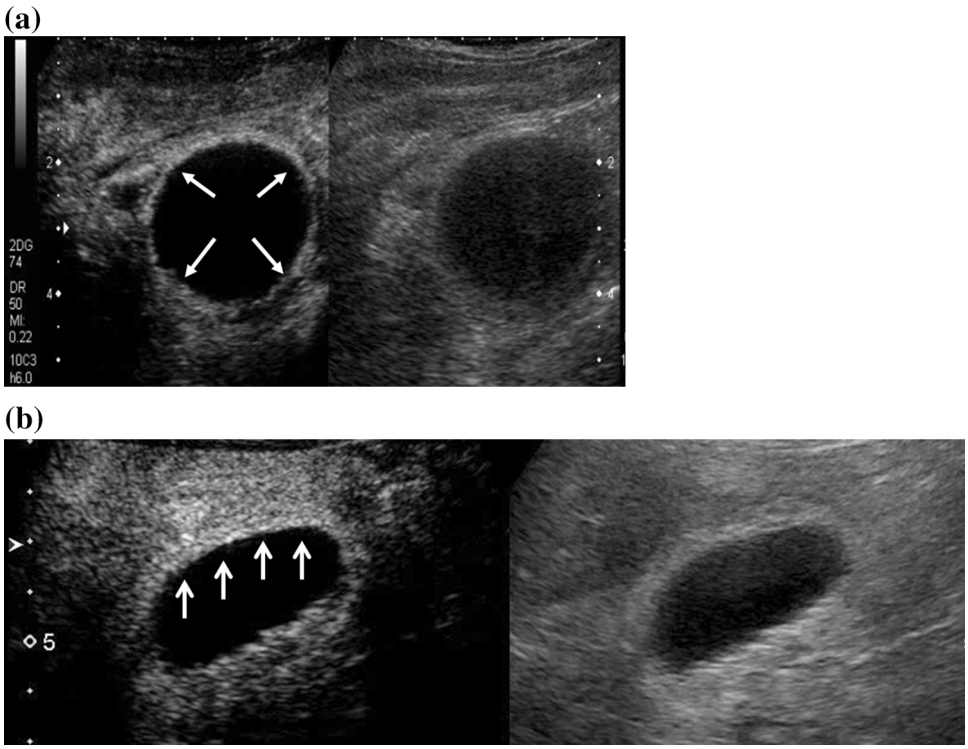

Fig. 1 Absence of perfusion defect (*arrows*) diagnosed as uncomplicated cholecystitis by CEUS with Sonazoid. Low-mechanical-index harmonic imaging with a gray-scale monitor (*right side*). **a** A case of uncomplicated cholecystitis (*short axis view*). **b** Another case of uncomplicated cholecystitis (*long axis view*)

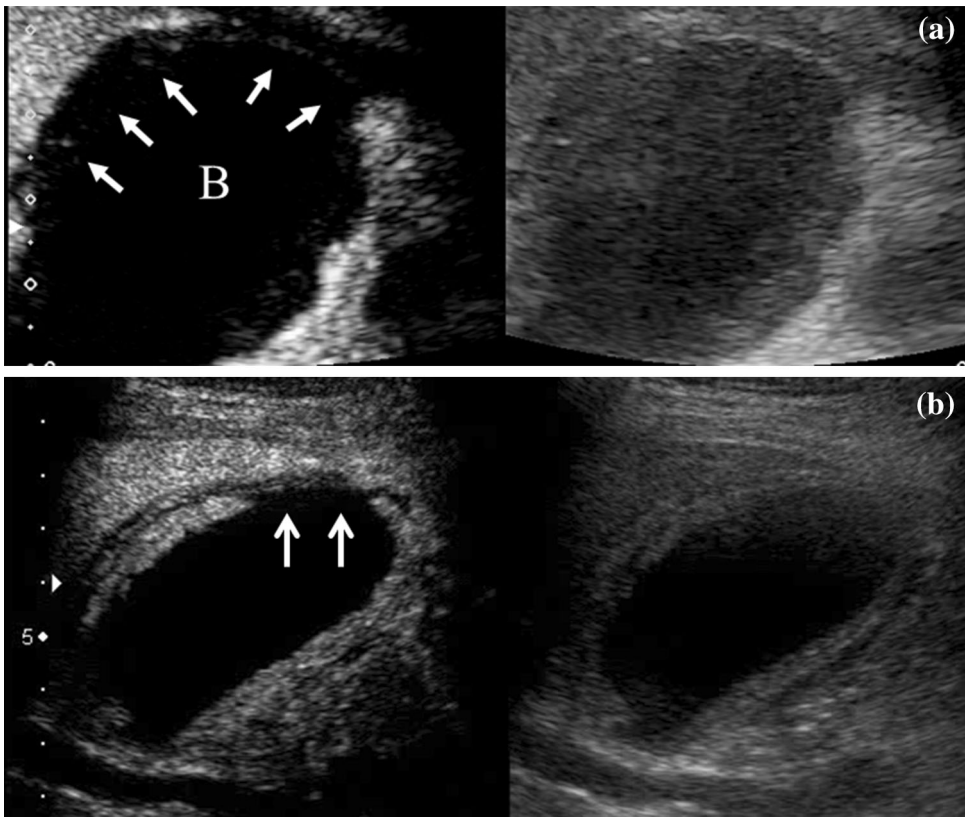

Fig. 2 Presence of perfusion defects (*arrows*) diagnosed as gangrenous cholecystitis by CEUS with Sonazoid. Low-mechanical-index harmonic imaging with a gray-scale monitor (*right side*). **a** A case of gangrenous cholecystitis. **b** Another case of gangrenous cholecystitis

age, white blood cell (WBC) count, C-reactive protein (CRP) level, proportion of correct diagnoses, time from onset of symptoms to US examination, time from US examination to surgery, gray-scale US findings, and proportion of patients with decreased signal intensity on CEUS. We evaluated the diagnostic accuracy using CEUS

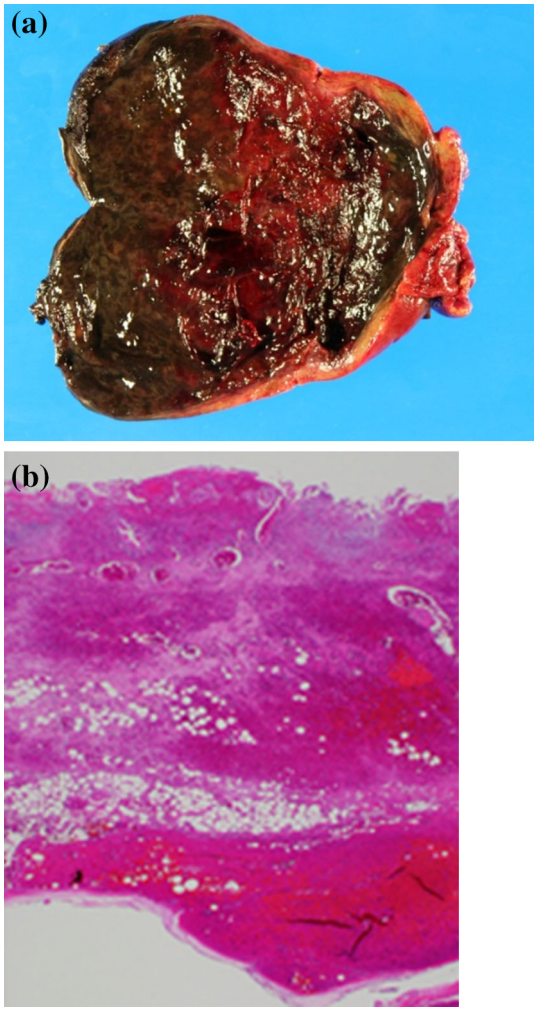

Fig. 3 Gangrenous cholecystitis. **a** Macropathological findings showing dark-colored mucosa and serous membrane. **b** Histological findings showing unclear structure of the gallbladder wall because of necrosis

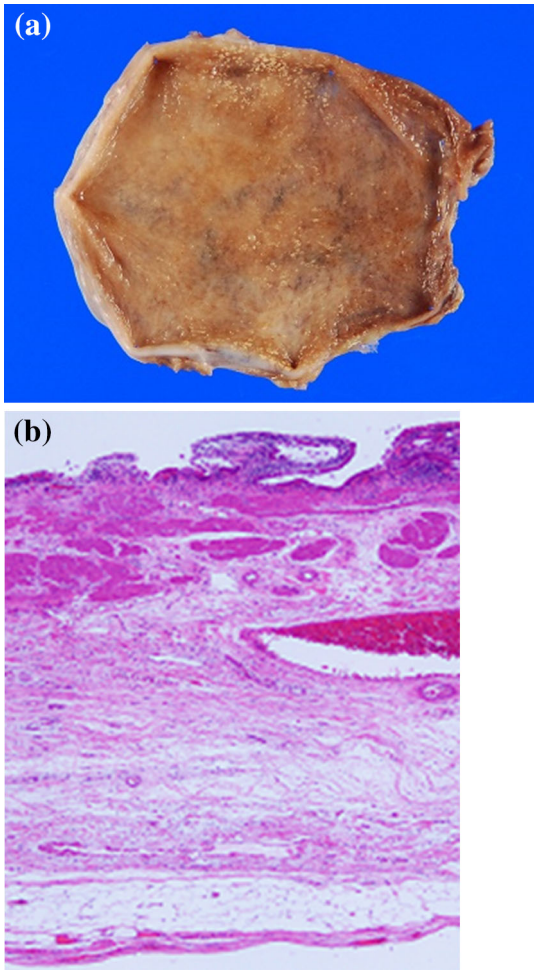

Fig. 4 Uncomplicated cholecystitis. **a** Macropathological findings showing light-brown-colored mucosa and serous membrane. **b** Pathological findings showing clear structure of the gallbladder wall without necrosis

by comparing the CEUS findings with the final diagnosis determined according to the histological findings; this comparison was performed using StatMate III for Windows version 3.19 (ATMS, Tokyo, Japan). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. The unpaired *t* test, Welch method, and χ^2 test with Yates compensation or Fisher's exact test were used to evaluate the significance of differences between groups. A p value <0.05 was considered to indicate a significant difference. Inter-observer agreement between one physician who performed the examination (preoperative diagnosis) and another physician who analyzed the movie clips was evaluated using the κ coefficient. A κ value of 0.0 indicated poor agreement, 0.01–0.20 slight agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 good agreement, and 0.81–1.0 excellent agreement.

Results

Seventy-four patients with suspected acute cholecystitis were investigated at our hospital. Among these 74 patients, 45 who provided informed consent to participate in this study underwent CEUS examination and were enrolled. However, 18 of 45 patients did not undergo cholecystectomy after CEUS examination and were excluded from the study. Thus, 27 patients (22 men and 5 women; mean age, 68.0 ± 15.4 years) who underwent surgery after CEUS were analyzed (Fig. 5). Of the 27 patients, 15 were in the GC group and 12 in the UC group. The characteristics of the patients in each group are shown in Table 1.

The GC group had a higher preoperative CRP level than the UC group ($p = 0.022$). The time from US examination to surgery was significantly shorter in the GC group than in the UC group ($p = 0.040$). Gray-scale US findings did not differ significantly between the two groups, except for the

prevalence of pericholecystic fluid collection. With respect to the CEUS findings, the presence of defects differed significantly between the two groups ($p = 0.0016$).

The usefulness of pericholecystic fluid collection and CEUS findings in the diagnosis of gangrenous cholecystitis are shown in Table 2. The presence of pericholecystic fluid collection had a sensitivity of 66.7 %, specificity of 83.3 %, PPV of 83.3 %, NPV of 66.7 %, and accuracy of 74.1 % for diagnosis of gangrenous cholecystitis. The physician who performed the examination found perfusion defects on CEUS in 10 of the 15 patients in the GC group, resulting in a sensitivity of 66.7 %, specificity of 100 %, PPV of 100 %, NPV of 70.6 %, and accuracy of 81.5 % for diagnosis of gangrenous cholecystitis. For the physician who subsequently reviewed the movie clips, these values were 73.3, 100, 100, 75.0, and 85.2 %, respectively. The inter-observer agreement between the physician who performed the examination and the physician who reviewed the movie clips was good (κ coefficient, 0.64).

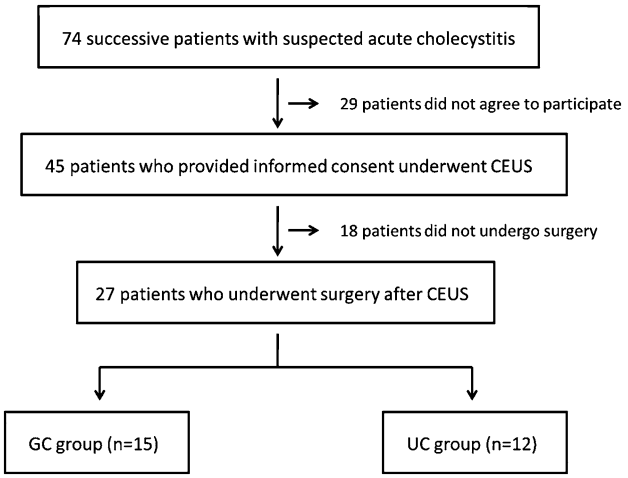

Fig. 5 Patient selection

Discussion

Gangrenous cholecystitis is a severe form of acute cholecystitis that results from marked distention of the gallbladder with increased tension in the wall. The associated inflammation leads to ischemic necrosis of the gallbladder wall with or without cystic artery thrombosis [12], differentiating it from uncomplicated cholecystitis. In our series, the definition of gangrenous cholecystitis included pathological micronecrosis, even in only part of the mucosal layer. The 27 patients analyzed in this study underwent cholecystectomy and might have had a more serious

Table 1 Characteristics of patients in the GC and UC groups

	GC Group	UC Group	*p*
Number of patients	15	12	
Male:female	14:1	8:4	0.203
Age in years	68.1 ± 15.7	68.0 ± 15.1	0.991
White blood cell count, /μL	12,707 ± 4895	10,885 ± 6263	0.418
C-reactive protein level, mg/dL	14.5 ± 7.5	5.8 ± 10.2	0.022
Diagnosis, correct:incorrect	10:5	12:0	0.086
Time from onset to US, days (range)	2.73 ± 2.63 (0–10)	3.58 ± 8.43 (0–30)	0.742
Time from US to surgery, days (range)	0.27 ± 0.458 (0–1)	16.58 ± 15.58 (1–60)	0.004
Gallbladder short axis, mm	39.39 ± 8.37	34.66 ± 4.94	0.081
Thickness of gallbladder wall, mm	5.75 ± 1.701	5.56 ± 1.874	0.787
Irregularity of the mucosal surface, *n* (%)	2 (13.3)	1 (8.3)	0.837
Gallbladder stone, *n* (%)	9 (60.0)	8 (66.7)	0.965
Sonographic Murphy sign, *n* (%)	11 (73.3)	10 (83.3)	0.877
Sonolucent layer, *n* (%)	10 (66.7)	8 (66.7)	0.681
Pericholecystic fluid, *n* (%)	10 (66.7)	2 (16.6)	0.027
CEUS perfusion defects, absence:presence	5:10	12:0	0.002

Table 2 Usefulness of each finding on ultrasonography for diagnosis of GC

	Sensitivity	Specificity	PPV	NPV	Accuracy
Pericholecystic fluid	0.667	0.833	0.833	0.667	0.741
Initial CEUS examination	0.667	1.0	1.0	0.706	0.815
Review of CEUS movie clips	0.733	1.0	1.0	0.750	0.852

clinical condition than the 18 patients who were excluded. Therefore, we consider that the proportion of patients with gangrenous cholecystitis (55.6 %, 15 of 27) was higher than that in previous reports (range, 2.0–29.6 %) [13].

Some researchers have reported that an elevated WBC count (>17,000/μL) in patients with acute cholecystitis suggests gangrenous cholecystitis [3, 13]. In our study, there was no significant difference in the WBC count between the GC and UC groups ($p = 0.418$); however, the CRP level was significantly higher in the GC group than in the UC group ($p = 0.022$). We consider that although these laboratory findings may provide some useful information, they are not necessarily diagnostic. It is therefore important to develop accurate imaging techniques for diagnosis of gangrenous cholecystitis.

Transabdominal US is regarded as a first-line noninvasive bedside investigation technique for the diagnosis of acute abdominal diseases [14], including acute cholecystitis [15, 16]. However, our findings suggest that it is difficult to differentiate between gangrenous and uncomplicated cholecystitis using only gray-scale US unless pericholecystic fluid collection is detected. This is partially consistent with the findings of other studies [17, 18]. One study [17] reported that thickening of the gallbladder wall and free fluid around the gallbladder were not specific for gallbladder inflammation in patients with cardiac failure, renal failure, hepatic cirrhosis, hepatitis, hypoalbuminemia, or blockage of lymphatic or venous drainage of the gallbladder. Another study [18] reported that only 33 % of patients with gangrenous cholecystitis exhibited the sonographic Murphy sign, possibly because of denervation. Conversely, 73.3 % of the patients (11 of 15) in the GC group in the present study exhibited a sonographic Murphy sign. This suggests that CEUS can detect necrosis before denervation. In our series, pericholecystic fluid collection also seemed useful for the diagnosis of gangrenous cholecystitis, which is not necessarily consistent with previous reports [17]. We did not analyze the findings of color Doppler examination, which also allows for the visualization of blood flow. This is because such an examination is generally unsuitable for the evaluation of microperfusion of the gallbladder mucosa, the flow velocity and signal power of which are often below the rejection level of the high-pass filter. For these reasons, we emphasize the need for CEUS for the evaluation of gallbladder ischemia.

CEUS with Sonazoid has been used in Japan since 2005. Sonazoid is a microbubble contrast agent that is metabolized by the liver and exhaled via the lungs. This agent has few serious adverse effects, except for infrequent allergic reactions, and can be safely used in patients with renal damage and at the bedside. Some studies have reported the usefulness of CEUS for various situations, including the diagnosis of mesenteric ischemia [19–21]. We therefore considered that CEUS may be useful for the diagnosis of many conditions in which we should evaluate microperfusion, such as necrosis of the gallbladder wall in patients with gangrenous cholecystitis.

In patients with acute inflammation of the gallbladder, CEUS seems to show increased contrast enhancement of the gallbladder wall [22–24]. Adamietz et al. [22] used CEUS with SonoVue to examine 20 patients with acute cholecystitis and 8 with chronic cholecystitis. They found that strong enhancement indicated acute inflammation, but low enhancement did not rule out acute cholecystitis, including two cases of gangrenous cholecystitis. We consider that reduced signal intensity or decreased perfusion on CEUS should be interpreted as gangrenous cholecystitis when the clinical and gray-scale US findings are highly suggestive of acute inflammation. To the best of our knowledge, only one previous study [11] used CEUS to differentiate between gangrenous and non-gangrenous cholecystitis. In that study, CEUS had a sensitivity of 83 % and specificity of 91 % for diagnosis of gangrenous cholecystitis. Our results showed a lower sensitivity (66.7–73.3 %) and higher specificity (100 %). The reasons for this difference are unclear, but may be related to the small number of patients in our study. We consider that our study and the above-mentioned previous study [11] strengthen the evidence regarding the usefulness of CEUS for diagnosing gangrenous cholecystitis.

In the present study, the diagnostic sensitivity by the physician who reviewed the movie clips was slightly greater than that by the physician who performed the examination. Repeated viewing of the movie clips without haste may improve the sensitivity of the findings.

There may be some disadvantages of CEUS. Inappropriate focusing of the ultrasound beams or the presence of acoustic shadows from stones can produce images mimicking flow defects seen in gangrenous cholecystitis. To avoid these misinterpretations, the focus should be adjusted properly, and the gallbladder wall should be scanned from various directions. Furthermore, it is sometimes difficult for inexperienced physicians to differentiate between microbubble contrast agent signals and tissue signals, which may result in gangrenous cholecystitis being interpreted as uncomplicated cholecystitis, thereby reducing the sensitivity of the examination. One should ensure that the signal from each microbubble is moving to discriminate it from the signals produced by the tissue.

Our study had some limitations. First, the number of cases may not have been large enough. Second, the time lag from US examination to surgery was significantly longer in the UC group than in the GC group ($p = 0.040$), which might have affected the diagnostic ability of our study if the gangrenous cholecystitis had been reversible.

Third, the surgeons were not blinded to the CEUS reports in our study, which might have influenced the decision-making process for emergency surgery. Finally, we did not evaluate the exact coincidence between the place and size of necrosis on histological examination and those of perfusion defects on CEUS because of the difficulty of strict comparison. However, the site of necrosis (gallbladder neck, body, or fundus) showed good coincidence (100 %, retrospectively) among the 10 cases of correctly diagnosed gangrene.

Conclusions

CEUS with Sonazoid is a sensitive and highly specific bedside modality for diagnosis of gangrenous cholecystitis, with good inter-observer agreement.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human rights statements and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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