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GASTROINTESTINAL

Quantitative contrast-enhanced US helps differentiating neoplastic vs non-neoplastic gallbladder polyps

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Abstract

Objectives To differentiate between large (≥ 1 cm in diameter) gallbladder (GB) non-neoplastic and neoplastic polyps using quantitative analysis of contrast-enhanced ultrasound (CEUS) findings.

Methods From September 2017 to May 2018, 29 patients (10 males; median age, 63 years) with GB polyps of ≥ 1 cm in diameter who were undergoing cholecystectomy were consecutively enrolled. All patients underwent preoperative conventional US and CEUS examinations. Quantitative analysis of CEUS findings using time-intensity curves between the two groups was independently performed by two radiologists. The interobserver agreement for the quantitative analysis of the CEUS results was measured using the intraclass correlation coefficient. Receiver operating characteristic analysis was performed to evaluate the diagnostic performance of CEUS examination.

Results After the cholecystectomy, the patients were classified into the non-neoplastic polyp group ($n = 12$) and the neoplastic polyp group ($n = 17$) according to the pathological results. The interobserver agreement for quantitative assessment between the two radiologists was near perfect to substantial. Quantitative assessment of the CEUS findings revealed that the rise time, mean transit time, time to peak, and fall time of non-neoplastic GB polyps were significantly shorter than those of neoplastic polyps ($p < 0.001$, $p = 0.008$, $p = 0.013$, and $p = 0.002$, respectively). The sensitivity and specificity of the quantitative CEUS parameters for the differentiation between the two groups were 76.5–100% and 75%, respectively, with an area under the curve of 0.765–0.887.

Conclusions Quantitative analysis of CEUS findings could be valuable in differentiating GB neoplastic polyps from non-neoplastic polyps.

Key Points

- *Quantitative analysis of CEUS findings could be valuable in differentiating gallbladder neoplastic polyps from non-neoplastic polyps.*
- *Quantitative analysis of CEUS findings in gallbladder polyps provides cut-off values for differentiation between neoplastic polyps and non-neoplastic polyps with near-perfect to substantial interobserver agreement.*

Keywords Contrast media · Ultrasonography · Gallbladder · Polyps

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Abbreviations

AUC	Area under the curve
CEUS	Contrast-enhanced ultrasound
FT	Fall time
GB	Gallbladder
MTT	Mean transit time
ROC	Receiver operating characteristic
ROI	Region of interest
RT	Rise time
TIC	Time-intensity curve
TTP	Time to peak enhancement

Introduction

Gallbladder (GB) polyps are a common condition with a prevalence of 5–7% in the adult population [1]. GB polyps comprise non-neoplastic polyps, such as cholesterol polyps, and neoplastic polyps, such as adenoma or adenocarcinoma [2]. Although non-neoplastic polyps constitute the majority of incidentally detected GB polyps, neoplastic polyps represent 8–13% of these polyps [3, 4], and the differentiation between these two types of GB polyps is a difficult task [5–7].

Currently, the management of GB polyps is mainly determined by its size [3, 8, 9]. Small polyps (< 1 cm in diameter) are followed up by ultrasound (US) unless associated with risk factors for malignancy, whereas laparoscopic cholecystectomy is routinely recommended for large polyps (≥ 1 cm in diameter). Neoplastic polyps should be resected because even adenomas, which are benign neoplastic lesions, have been reported to progress into malignancy in approximately 30% of cases [10, 11]. However, not all GB polyps of ≥ 1 cm in diameter are neoplastic polyps [5, 12]. According to the current guidelines, the patients with non-neoplastic GB polyps of ≥ 1 cm in diameter undergo futile cholecystectomy, which is associated with morbidity including persistent abdominal pain in ≤ 20% and bile duct injury in ≤ 1.7% of patients [13, 14]. Therefore, an accurate characterization of GB polyps is imperative to avoid unnecessary surgery and surgical complications in patients with non-neoplastic GB polyps of ≥ 1 cm in diameter.

Conventional gray-scale US is the primary imaging technique of GB [15, 16]. However, differentiation between non-neoplastic and neoplastic polyps by a gray-scale US alone is not sufficient [17]. The diagnostic performance of color Doppler US in the diagnosis of GB polyps is disappointing due to its low sensitivity for slow blood flow and blooming or overpainting artifacts [18–21].

Contrast-enhanced ultrasound (CEUS), which uses purely intravascular microbubble contrast agents, allows for visualization of lesion perfusion and is widely used in abdominal organs such as the liver, kidney, and pancreas [22, 23]. Microbubble contrast agent has no renal toxicity and can be safely administered in patients with decreased renal function [24]. Previous studies investigated CEUS in the diagnosis of GB disease including polyps and carcinoma [25–30]. Unfortunately, these studies considered only qualitative analysis of the CEUS images and were therefore subjective and prone to interobserver variation. To overcome these limitations and fully utilize the CEUS information, quantitative assessment including time-intensity curve (TIC) analysis is strongly warranted. Therefore, the purpose of our study was to attempt distinguishing non-neoplastic from neoplastic GB polyps of ≥ 1 cm in diameter using quantitative analysis of CEUS results.

Materials and methods

Our single-institutional and prospective study was performed in compliance with the Health Insurance Portability and Accountability Act. The Institutional Review Board of our hospital approved this study (H-1806-120-952) and written informed consent was obtained from all patients.

Patients

Between September 2017 and May 2018, 36 consecutive patients with GB polyps were recruited via the outpatient clinics of the Department of Internal Medicine and Surgery in our hospital. All 36 patients were examined using both conventional US and CEUS. The indications for cholecystectomy in our hospital were GB polyps of ≥ 1 cm in diameter or the presence of symptoms such as abdominal pain or dyspepsia. After US examination, four patients did not undergo cholecystectomy and were excluded from the study. Finally, 32 patients (11 males and 21 females; median age, 63 years) with a confirmative pathological diagnosis of GB polyps were enrolled in our study (Fig. 1).

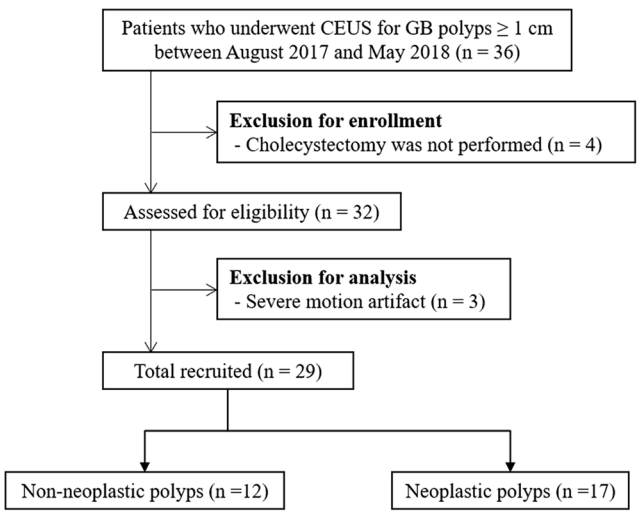


Fig. 1 Flow diagram of our study population. CEUS, contrast-enhanced ultrasound; GB, gallbladder

US examination

After fasting for more than 6 h, conventional US and CEUS examinations were conducted using a US system (LOGIQ E9, GE Healthcare) with a 2–9 MHz convex probe. All US examinations were performed by an experienced radiologist (with 22 and 18 years of clinical experience in conventional US and CEUS, respectively) to avoid interobserver variability. Patients were requested to hold their breath or to breathe shallowly in order to acquire good and stable imaging planes. If there were multiple polyps, the largest polyp was selected for the examination. First, conventional gray-scale US and color Doppler US examinations of the GB polyp were performed. Thereafter, CEUS was performed with an intravenous injection of a single bolus of 2.4 mL of suspension of stabilized sulfur hexafluoride-filled microbubbles (SonoVue®, Bracco) followed by a 10 mL 0.9% saline flush. CEUS examinations were performed using the following parameters that were maintained the same before and after injection of the contrast agent: mechanical index, 0.08; 10–13 frames per second; dynamic range, 65 dB; signal persistence turned on; echo-signal gain below noise visibility; power modulated pulse inversion technology; and one focus at the GB polyp. The CEUS examination required ≤3 min to perform and was recorded as a video clip for subsequent quantitative analysis.

US image analysis

Two radiologists (with 6 and 7 years of experience in CEUS imaging, respectively) independently assessed the conventional US, including color Doppler US, and CEUS images in qualitative and quantitative manners. They were blinded to the results of other imaging modalities and the pathological diagnosis of GB polyps. The detailed information on evaluation of conventional US images and qualitative evaluation of CEUS images are described in the Supplementary material.

For quantitative analysis of the CEUS images, the video clips were downloaded from the US machine and transferred to a different computer for independent assessment by the two radiologists. Commercialized perfusion analysis software (VueBox®, Bracco) was used to assess the CEUS images. Before calculation, automatic motion correction was used, and the out-of-plane images primarily caused by respiratory movements of the patients were excluded from the analysis. A manually defined region of interest (ROI) was placed on the GB polyp on each image and the averaged echo intensity in the ROI was linearized and normalized and was converted to the microbubble concentration. For some GB polyps that required full inspiration or expiration for optimal visualization, a respiratory motion was inevitable during the acquisition of video clips. We attempted to draw the ROI as large as possible while maintaining its location within the GB polyp and minimizing the effect of respiratory motion. A second ROI was drawn in the adjacent normal hepatic parenchyma to serve as an internal reference for the adequacy of perfusion analysis. This reference ROI was drawn at the same depth as the other ROI for the GB polyp and care was taken to avoid artifacts. The microbubble concentration in each ROI was plotted against the time to yield a TIC, and curve fitting analysis was provided by the VueBox® software (Fig. 2). From the TIC of each patient, the following kinetic parameters were automatically calculated: PE; area under the curve (AUC) during wash-in (WiAUC), wash-out, and the sum of both; rise time (RT); mean transit time (MTT); time to PE (TTP); wash-in and wash-out rate; and wash-in perfusion index (WiPI, WiAUC divided by RT) [31]. The quality of fit between the theoretical curve and the echo-power signal was also quantified.

After independent quantitative analysis of the CEUS images by the two radiologists, the interobserver agreement was assessed. Thereafter, a consensus was reached by the two radiologists for the placement of the ROI. Discrepancies between the two reviewers were resolved through discussion with a third radiologist (with 18 years of clinical experience in CEUS imaging).

Clinical and pathologic analysis

Clinical information including symptoms and preoperative serum level of carcinoembryonic antigen (CEA) and

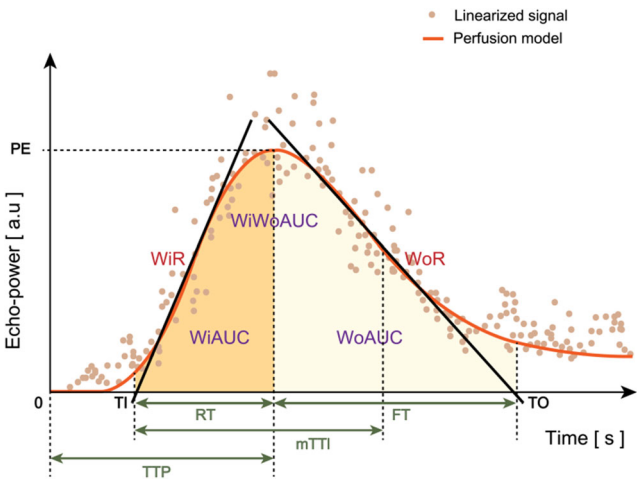


Fig. 2 The perfusion parameters obtained from the quantitative analysis of the CEUS examination. PE, peak enhancement (a.u); TTP, time to peak (s); WiAUC, wash-in area under the curve (AUC, TI:TTP) (a.u); RT, rise time (TTP – TI) (s); MTT, mean transit time (MTT – TI) (s); WiR, wash-in rate (maximum slope) (a.u); WiPI, wash-in perfusion index (WiAUC/RT) (a.u); WoAUC, wash-out AUC (TTP:TO) (a.u); WiWoAUC, wash-in and wash-out AUC (WiAUC+WoAUC) (a.u); FT, fall time (TO – TTP) (s); WoR, wash-out rate (minimum slope) (a.u); QOF, quality of fit between the echo-power signal and *f*(*t*) (%); TI, the instant at which the maximum slope tangent intersects the *x*-axis; TO, the instant at which the minimum slope tangent intersects the *x*-axis

carbohydrate antigen (CA) 19-9 was collected. The final pathological diagnosis of GB polyps in the surgical specimen was made by an experienced pathologist (with 18 years of experience in biliary pathology) who was blinded to the clinical information and the results of imaging investigations including the CEUS images. The size and T stage (in cases of malignant polyps) of GB polyps were assessed. For the determination of correlations with perfusion characteristics on CEUS, a meticulous retrospective assessment for the vascularity of the polyps was also performed. After pathological evaluation, GB polyps were classified into non-neoplastic polyps and neoplastic polyps. The clinical and histopathological characteristics of the patients are provided in Table 1.

Statistical analysis

Univariate analysis was performed using the Mann-Whitney *U* test for continuous variables and the chi-square test or Fisher's exact test for categorical variables to compare the clinical, US, and CEUS characteristics between patients with non-neoplastic and neoplastic polyps. The factors with $p < 0.05$ were considered statistically significant. Thereafter, the diagnostic performance of CEUS parameters that exhibited significant differences was assessed using receiver operating characteristic (ROC) analysis. Interobserver agreement for qualitative and quantitative analysis was assessed using weighted κ statistics for categorical variables and the intraclass correlation coefficient (ICC) for continuous variables. The following convention was used to interpret the values of weighted κ statistics and the ICC: < 0.20, poor; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, substantial; and 0.81–1.00, near-perfect agreement [32]. For the quantitative assessment of the CEUS results, we performed a subgroup analysis for GB polyps of ≤ 2 cm in diameter because the differentiation between non-neoplastic and neoplastic polyps is particularly challenging for such borderline-sized polyps [33]. All statistical analyses were conducted using commercial statistical software (SPSS Statistics for Windows, version 23, IBM and MedCalc, version 18.2.1).

Results

Among the 32 patients who underwent preoperative US and CEUS as well as cholecystectomy, three patients were excluded from the analysis due to severe motion artifacts that impeded the quantitative analysis. Therefore, 29 patients were included in the final analysis (10 males and 19 females; median age, 63 years; age range, 28–85 years). There were 12 non-neoplastic polyps and 17 neoplastic polyps. Non-neoplastic polyps were cholesterol polyps in 10 patients and inflammatory polyps in two patients. Neoplastic polyps consist of adenocarcinoma ($n = 12$), intracystic tubulopapillary neoplasm ($n = 2$), adenoma ($n = 2$), and adenosquamous carcinoma ($n = 1$). The clinical characteristics of the patients are provided in Table 1. The patients with neoplastic polyps were significantly older and had a significantly higher level of preoperative tumor markers than did the patients with non-neoplastic polyps ($p = 0.001$, $p = 0.014$, and $p = 0.046$, respectively) (Table 1).

In terms of qualitative analysis for the conventional US and CEUS features, the neoplastic polyps were significantly larger and more likely to be sessile morphology than the non-neoplastic polyps ($p < 0.001$ and $p = 0.002$, respectively) (Table 2). Neoplastic polyps also tended to have spotty or linear vascularity on color Doppler US while non-neoplastic polyps did not exhibit any vascularity ($p = 0.001$) (Table 2).

Quantitative analysis of CEUS parameters revealed that the RT, MTT, TTP, and FT were significantly shorter in non-neoplastic polyps than in neoplastic polyps ($p < 0.001$, 0.008, 0.013, and 0.002, respectively) (Table 3) (Fig. 3). In terms of the diagnostic performance of the CEUS parameters that showed statistical significance in the univariate analysis, the sensitivity and specificity for distinguishing neoplastic polyps from non-neoplastic polyps ranged from 76.5 to 100.0% and 75.0%, respectively, with near-perfect to substantial agreement (Tables 3 and 4).

Table 1 Clinical characteristics of 29 study patients

	Non-neoplastic polyps ($n = 12$)	Neoplastic polyps ($n = 17$)	*p* value
Sex (male:female)	2:10	8:9	0.126
Age (years)	51.0 (28–69)	67.0 (52–85)	0.001
Symptoms*	1 (8.3)	4 (23.5)	0.370
Time interval† (days)	21.5 (1–166)	3.0 (1–55)	0.301
CA 19–9 (unit/mL)	2.9 (0.5–14)	11.2 (0.9–40.9)	0.014
CEA (ng/mL)	1.1 (0.7–3.6)	1.9 (0.7–15.7)	0.046

Values are median, and numbers in parentheses are ranges unless specified

*Numbers in parentheses are percentages

† Time interval between US examination and surgery

CA 19-9, carbohydrate antigen 19-9; *CEA*, carcinoembryonic antigen

Table 2 Results for qualitative analysis of conventional US and CEUS characteristics in 29 study patients

	Non-neoplastic polyps ($n = 12$)	Neoplastic polyps ($n = 17$)	p value	Agreement
Conventional US features				
Size (cm)*	1.1 (0.8–1.5)	2.4 (1.2–11.0)	< 0.001	0.134
Number of polyp single:multiple	11:1	15:2	> 0.999	< 0.001
Morphology of polyp pedunculated:sessile	11:1	5:12	0.002	0.859
Echogenicity of polyp hypo:iso:hyper	8:3:1	8:8:1	0.610	0.372
Vascularity of polyp absent:spotty:linear	3:2:1	2:6:8	0.010	0.060
Echogenic foci in the polyp absent:present	11:1	17:0	0.414	< 0.001
Integrity of the GB wall intact:disrupted	12:0	12:5	0.052	< 0.001
CEUS features				
Contrast arrival time (s)*	15.0 (8.0–31.0)	15.0 (5.0–27.0)	0.343	< 0.001
Vascular type of the polyp homogeneous dotted:single vessel:branch-like:tortuous or irregular	6:4:1:0	12:1:2:2	0.132	< 0.001
Enhancement pattern of the polyp eccentric:diffuse	11:0	16:1	0.665	< 0.001
Peak time appearance of the polyp homogeneous:heterogeneous	10:1	12:5	0.180	0.087
Extent of peak enhancement of the polyp hypo:iso:hyper	1:2:9	0:1:16	0.364	< 0.001
Wash-in timing of the polyp slow:synchronous:fast	1:1:10	0:2:15	0.742	< 0.001
Wash-out timing of the polyp slow:synchronous:fast	1:4:7	0:3:14	0.266	0.047
Integrity of the GB wall intact:disrupted	11:0	16:1	0.665	< 0.001

*Values are median, and the numbers in parentheses are ranges

CEUS, contrast-enhanced ultrasound; *GB*, gallbladder

For the subgroup analysis of GB polyps of ≤ 2 cm in diameter, all non-neoplastic polyps were ≤ 2 cm in diameter while only 29.4% (5/17) of neoplastic polyps were ≤ 2 cm in diameter. In this subgroup analysis, the same variables as those in the entire GB polyps analysis significantly differed between non-neoplastic and neoplastic polyps (RT, $p = 0.001$; MTT, $p = 0.028$; TTP, $p = 0.033$; and FT, $p = 0.006$).

The pathology slides of all polyps were retrospectively reviewed for meticulous assessment of the microvasculature and the nature of the stroma. The supply vessels of the non-neoplastic polyps were muscular vessels, which were more numerous and more dilated than those of the adjacent GB lamina propria. In contrast, the supply vessels of the neoplastic polyps were smaller in caliber and less conspicuous than those of the

Table 3 Results of quantitative analysis for time-intensity curves derived from CEUS examinations

	Non-neoplastic polyps ($n = 12$)	Neoplastic polyps ($n = 17$)	p value	Agreement
Peak enhancement (a.u)	1228.9 (197.4–19,567.0)	1226.1 (61.2–7346.5)	0.929	0.820
Wash-in AUC (a.u)	3363.4 (426.1–61,628.1)	8221.3 (218.9–28,735.9)	0.170	0.724
Rise time (s)	5.1 (2.1–8.3)	8.3 (5.0–17.4)	*< 0.001*	0.843
Mean transit time (s)	19.1 (3.0–19.1)	35.9 (13.2–110.6)	*0.008*	0.445
Time to peak (s)	7.9 (3.7–23.3)	12.8 (5.4–38.8)	*0.013*	0.919
Wash-in rate (a.u)	471.4 (38.7–5447.6)	238.1 (11.2–1800.6)	0.215	0.915
Wash-in perfusion index (a.u)	791.3 (119.9–12,327.1)	781.8 (42.2–4655.2)	0.965	0.826
Wash-out AUC (a.u)	6934.4 (634.9–103,377.8)	14,460.3 (694.6–47,259.8)	0.132	0.772
Wash-in and wash-out AUC (a.u)	9908.9 (1061.0–165,005.9)	22,402.0 (1004.8–75,995.8)	0.144	0.754
Fall time (s)	8.9 (3.4–26.9)	17.4 (10.9–40.9)	*0.002*	0.774
Wash-out rate (a.u)	175.4 (10.6–2437.0)	90.5 (3.0–753.2)	0.121	0.856
Quality of fit (%)	62.7 (37.0–87.1)	73.5 (3.9–94.5)	0.170	0.674
Area of the ROI (mm^2)	8.8 (2.8–46.9)	10.2 (5.5–23.3)	0.364	0.751

Values are median, and numbers in parentheses are ranges

CEUS, contrast-enhanced ultrasound; *a.u*, arbitrary unit; *AUC*, area under the curve; *ROI*, region of interest

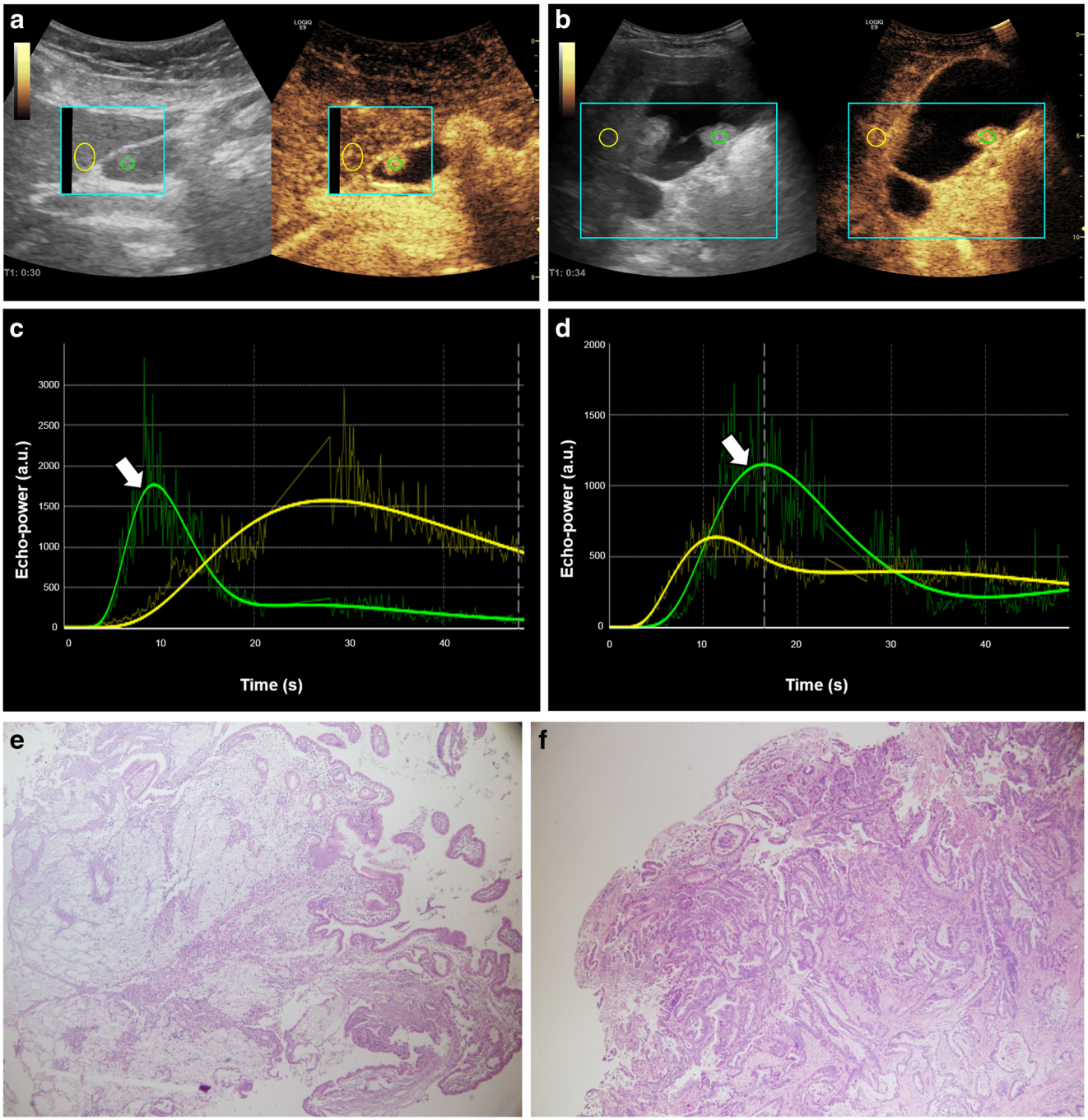


Fig. 3 Contrast-enhanced ultrasound (CEUS) examinations in a 28-year-old male patient with a 1.0-cm non-neoplastic gallbladder (GB) polyp (**a**, **c**, **e**) and a 78-year-old male patient with a 1.5-cm neoplastic GB polyp (**b**, **d**, **f**). On CEUS examination, both non-neoplastic polyp (**a**) and neoplastic polyp (**b**) showed homogeneous enhancement (arrows) after injection of contrast agent. A green region of interest was placed in the GB polyp and a yellow region of interest was placed in the adjacent hepatic parenchyma at the different level as the GB polyp. On the time-intensity curve acquired through quantitative analysis of the CEUS examination, green (arrow) and yellow curves were fitted to the echo-power signal corresponding to the GB polyp and hepatic parenchyma, respectively. The time-intensity curve revealed that the rise time (RT) and fall time (FT) of the non-neoplastic polyp (5.1 s and 8.7 s, respectively) (**c**) were shorter than those of the neoplastic polyp (9.9 s and 18.4 s, respectively) (**d**). After surgery, the polyp with shorter RT and FT was diagnosed as a cholesterol polyp (**e**) and the polyp with longer RT and FT was confirmed to be an adenocarcinoma (**f**). (**e**, **f**: Hematoxylin and eosin stain, original magnification × 10)

non-neoplastic polyps. While microvascular assessment could be performed without difficulty in the non-neoplastic polyps due to the sparse cellularity and loose stroma, identifying vessels in neoplastic polyps purely based on morphology was more challenging due to the dense tumor cellularity, relatively sparse stroma, and compressed vascular structures (Fig. 4).

Table 4 Diagnostic performances of the CEUS parameters in differentiation of non-neoplastic polyps from neoplastic polyps

CEUS parameters	Cut-off value (s)	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Rise time	5.7	0.892 (0.721, 0.976)	94.1 (71.3, 99.9)	75.0 (42.8, 94.5)	84.2 (66.5, 93.5)	90.0 (56.7, 98.4)	86.2 (25/29)
Mean transit time	23.6	0.794 (0.604, 0.921)	88.2 (63.6, 98.5)	75.0 (42.8, 94.5)	83.3 (64.9, 93.1)	81.8 (54.0, 94.5)	82.8 (24/29)
Time to peak	9.6	0.775 (0.582, 0.908)	76.5 (50.1, 93.2)	75.0 (42.8, 94.5)	81.3 (61.1, 92.3)	69.2 (47.3, 84.9)	75.9 (22/29)
Fall time	9.8	0.848 (0.666, 0.953)	100.0 (80.5, 100.0)	75.0 (42.8, 94.5)	85.0 (68.0, 93.8)	100.0	89.7 (26/29)

CEU, contrast-enhanced ultrasound; *AUC*, area under the curve; *PPV*, positive predictive value; *NPV*, negative predictive value

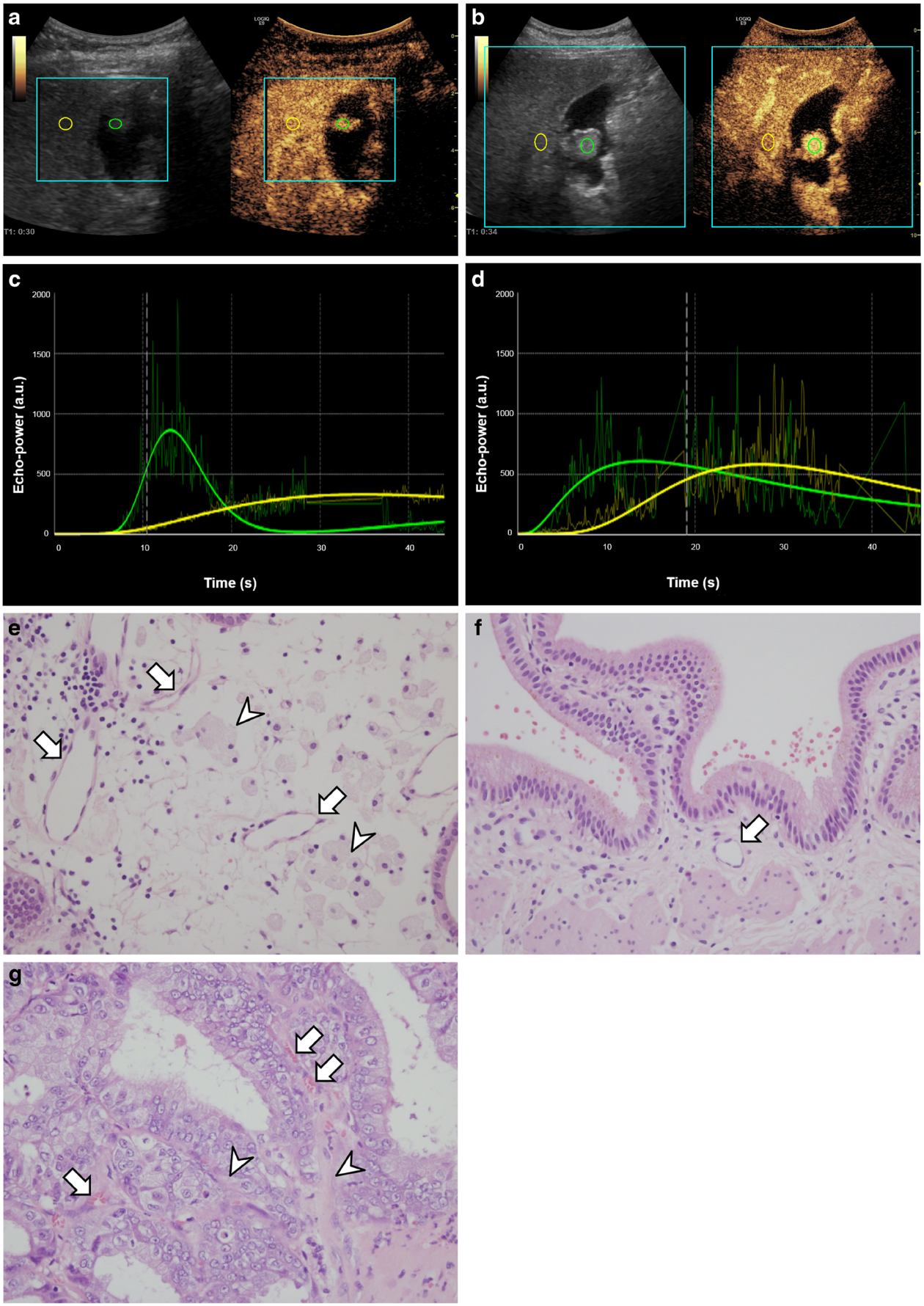
Discussion

In our study, quantitative analysis of CEUS findings using TIC analysis revealed that non-neoplastic GB polyps had a significantly shorter RT, MTT, TTP, and FT than did neoplastic polyps with a sensitivity of 76.5–100.0% and specificity of 75%. This observation may be somewhat contradictory to the common belief that malignant lesions tend to exhibit faster wash-in and wash-out than do benign lesions on contrast-enhanced dynamic studies including CEUS, particularly in the liver [22], as well as to the previous literature [26]. Therefore, we performed a meticulous pathological analysis to identify the pathological background explaining our observation and the apparent paradox. Upon histopathological review, non-neoplastic polyps, particularly cholesterol polyps, demonstrated increased stromal microvasculature compared to that of the adjacent lamina propria, with dilatation of the vascular lumen. In contrast, although neoplastic polyps have increased vascularity due to angiogenesis [34], the vessels were smaller in caliber and less conspicuous than those of non-neoplastic polyps. Such histological characteristics may cause significant differences in time-related CEUS parameters such as the RT, MTT, TTP, or FT on TIC analysis between the two polyp groups. Furthermore, the difference in perfusion characteristics may be partially explained by the different vascular system of the GB compared to that of the liver, which receives dual blood supply from the hepatic artery and portal vein. On the contrary, the GB is supplied only by the cystic artery and drains into peripheral portal venous branches [35]. Although an early wash-out of contrast agent was more frequently observed in GB carcinomas (91%) than in benign GB lesions (17%) in a previous study [26], the investigators did not perform a quantitative analysis of contrast agent kinetics. Therefore, the evaluation of wash-out on CEUS images may be subjective and could be influenced by the enhancement of the adjacent hepatic parenchyma. According to our study results, we believe that a significant proportion of patients with GB non-neoplastic polyps of ≥ 1 cm in diameter could avoid an unnecessary cholecystectomy.

In general, a lack of reproducibility is one of the major obstacles for quantitative assessment in imaging studies including CEUS. However, the inter-reader agreements in our study were near perfect to substantial to aside from that for MTT, which was similar to that of previous studies on quantitative analysis of CEUS performed on other organs [36, 37]. In this study, we used a motion correction algorithm before defining the ROI on the polyps in all patients. Given that respiratory motion significantly and negatively impacts the accuracy of quantitative values, we believe that the use of a motion correction algorithm is mandatory in quantitative assessment for abdominal diseases. Indeed, a quality of fit indicating the appropriateness of TIC analysis significantly improved when a motion correction algorithm was applied [38]. In addition, we specifically instructed the patients regarding their respiration control before the CEUS examination was performed. The use of a motion correction algorithm and specific respiratory instructions may be responsible for the high inter-reader agreements regarding the CEUS parameters in our study.

In clinical practice, there is no debate regarding the management of apparently neoplastic GB polyps manifesting as a very large (≥ 2 cm in diameter) mass or exhibiting clear disruption of the GB wall. However, there is controversy regarding the management of "borderline-size" GB polyps that are ≥ 1 cm but < 2 cm in diameter [33, 39]. In a subgroup analysis

Fig. 4 Representative examples of non-neoplastic and neoplastic gallbladder (GB) polyps. Contrast-enhanced ultrasound (CEUS) examinations in a 58-year-old female patient with a 1.0-cm non-neoplastic GB polyp (**a**, **c**, **e**, **f**) and a 63-year-old female patient with a 2.1-cm neoplastic GB polyp (**b**, **d**, **g**). **a**–**d** CEUS images and time-intensity curves demonstrated typical enhancement pattern and kinetics patterns for a non-neoplastic polyp and a neoplastic polyp. **a**, **c** The non-neoplastic polyp showed a homogeneous enhancement after injection of contrast media and a rise time of 5.2 s and a fall time of 7.5 s. **b**, **d** The neoplastic polyp showed homogeneous enhancement after injection of contrast agent as well. However, quantitative analysis yielded a rise time of 11.8 s and a fall time of 40.9 s. **e, f** The stroma of the cholesterol polyp is abundant and loose compared to the lamina propria of the gallbladder and contains lipid-laden macrophages (arrowheads) and scattered lymphocytes. The vasculature (arrows)—mainly characterized by thin-walled vessels—in the stroma of the cholesterol polyp is more numerous compared to the lamina propria and are dilated. **g** In contrast, neoplastic polyps (adenocarcinoma in this photomicrograph) are densely packed with neoplastic epithelium with relatively sparse stroma (arrowheads). The microvasculature (arrows) is compressed and not easily discernible due to the small caliber. (**e**, **f**, **g**: Hematoxylin and eosin stain, original magnification × 400)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of GB polyps of < 2 cm in diameter, a similar trend to that of all GB polyps was found. Specifically, non-neoplastic GB polyps had a significantly shorter RT, MTT, TTP, and FT than did neoplastic polyps of < 2 cm in diameter. Although the sample size was relatively small, particularly for neoplastic polyps of < 2 cm in diameter ($n = 5$), we found that our results might be applicable for those "borderline-size" GB polyps.

We also found that there was no difference between the two groups regarding the qualitative items of CEUS examination, while a quantitative analysis using a TIC of the CEUS examinations revealed significant differences between the two polyp groups. This does not coincide with previous studies in which several qualitative CEUS features including heterogeneous enhancement of GB polyps or GB wall destruction were useful for the differentiation of neoplastic GB polyps from non-neoplastic polyps [20, 26, 40]. This discrepancy probably reflects the subjective nature of qualitative analysis and its vulnerability to reproducibility.

There are several limitations in our study. First, the sample size was small ($n = 29$), particularly for the number of GB polyps of < 2 cm in diameter ($n = 17$). However, we designed this study as a preliminary study investigating the feasibility of quantitative CEUS analysis for the distinction of GB polyps. Second, because we used a single type of contrast agent (SonoVue®), generalization of our results to other US contrast agents requires caution, although other US contrast agents are expected to behave in the same way. Third, because the assessment of the supplying microvasculature was performed on hematoxylin and eosin stained slides alone, immunohistochemical stains for endothelial cell markers (e.g., CD34) would be necessary for a more accurate quantitative assessment of vascularity. Fourth, we applied a single ROI for the quantification of the perfusion of GB polyps. However, we thought that volumetric analysis using the volume of interest (VOI) would enable better assessment of the perfusion of GB polyps although that feature is not provided by the software (VueBox®). Last, quantitative analysis of CEUS data was performed using the results obtained by a single US scanner and a special commercial perfusion software (VueBox®). Therefore, further validation through future investigations using various US machines and perfusion software programs is warranted.

In conclusion, a quantitative analysis of CEUS using a TIC may be useful for the differentiation between non-neoplastic and neoplastic GB polyps of ≥ 1 cm in diameter. Our results may have the potential to decrease the number of unnecessary cholecystectomies for patients with large (≥ 1 cm in diameter) non-neoplastic polyps.

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Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Joon Koo Han.

Conflict of interest The authors of this manuscript declare no relationships with any companies, whose products or services may be related to the subject matter of the article.

Statistics and biometry No complex statistical methods were necessary for this paper.

Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Methodology
- prospective
- diagnostic or prognostic study
- performed at one institution

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