

## Original Article

# Conventional ultrasound and contrast-enhanced ultrasound in evaluating the severity of Crohn's disease

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**Abstract:** Objective: To evaluate the value of conventional ultrasound and contrast-enhanced ultrasound (CEUS) in determining the severity of active Crohn's disease. Methods: Thirty-seven patients who were considered to be in active period of Crohn's disease were included. Conventional ultrasound was employed to measure the thicknesses of interior, exterior and the whole bowel walls. Qualitative and quantitative CEUS analysis of the interior, exterior and the whole intestinal walls were also performed. Correlations between these methods and the severity of Crohn's disease were assessed. Results: Endoscopy grading system identified 19 patients with mild disease and 18 with severe disease. In discriminating severe Crohn's disease from mild disease, the cut-off value for the thickness of the entire bowel wall was 6.8 mm by receiver operating characteristic (ROC) analysis, with area under ROC (AUROC) of 0.84, sensitivity of 94.4%, specificity of 68.4%, positive predictive value (PPV) of 61.1%, negative predictive value (NPV) of 69.2%, and Youden's index of 0.628. The cut-off value for thickness of the interior intestinal wall was 4.8 mm (AUROC, 0.81; sensitivity, 88.9%; specificity, 63.2%; PPV, 85.7%; NPV, 69.6%; Youden's index, 0.521). The sensitivity, specificity, PPV, NPV, accuracy, and Youden's index of CEUS qualitative analysis were 100% (18/18), 57.9% (11/19), 64.3% (18/26), 100% (11/11), 78.4% (29/37), and 0.579, respectively. Quantitative comparison revealed that patients with mild disease and those with severe disease differed only in I<sub>max</sub> of inner bowel wall enhancement (2746.9 ± 911 vs. 12814.5 ± 9802.4; *P* = 0.02) and I<sub>max</sub> of entire wall enhancement (2106 ± 660 vs. 9864 ± 6994; *P* = 0.03). The cut-off value for the I<sub>max</sub> of the entire bowel wall was 3067, with the AUROC of 0.96, sensitivity of 100%, specificity of 67.7%, PPV of 100%, NPV of 88.9%, and Youden's index of 0.677; and the cut-off value for the I<sub>max</sub> of the interior intestinal layer was 3356, with the AUROC of 1.00, sensitivity of 100%, specificity of 100%, PPV of 100%, NPV of 100%, and Youden's index of 1.0. Conclusions: Both conventional ultrasound and CEUS are reliable methods in determining the severity of active Crohn's disease. The diagnostic performance in terms of Youden's index was highest for the I<sub>max</sub> of the interior layer, in comparison with all other features on conventional ultrasound, qualitative CEUS, and quantitative CEUS.

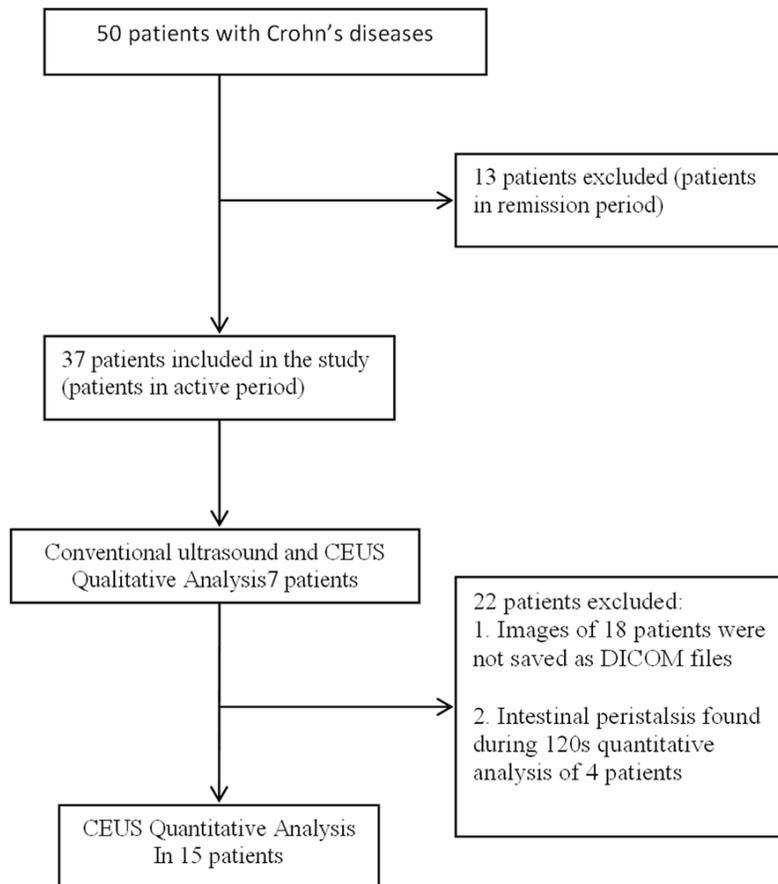
**Keywords:** Crohn's disease, conventional ultrasound, contrast-enhanced ultrasound, qualitative analysis, quantitative analysis

## Introduction

Crohn's disease is a chronic transmural intestinal inflammatory disease characterized by episodes of inflammation alternating with periods of remission [1-10]. Assessment of disease severity is crucial to guide the therapeutic strategy and establish prognosis. The severity of Crohn's disease is often assessed by clinical symptoms using Crohn's disease activity index (CDAI) [5], which is considered as a reference to determine the activity of Crohn's disease.

However, patients with mild disease often have high CDAI scores because CDAI has low specificity and it's based on subjective symptoms [4]. On the other hand, endoscopy of terminal ileal loops, computed tomography (CT) and magnetic resonance imaging (MRI) were considered as elective methods to evaluate the activity of Crohn's disease [1-3]. Endoscopy can give direct information of the inner mucosal layer, thus it allows more objective and reliable measurement of the activity of Crohn's disease; and it is considered as the gold standard for severity

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**Figure 1.** Flow diagram of study group.

**Table 1.** Basic characteristics of the patients with Crohn's disease

	Mild Crohn's disease	Severe Crohn's disease
Patient number (n = 37)	19	18
Mean age (years, range)	33 ± 12 (18-65)	36 ± 13 (19-66)
Gender		
Male, N (%)	11 (57.9)	9 (50)
Female, N (%)	8 (42.1)	9 (50)

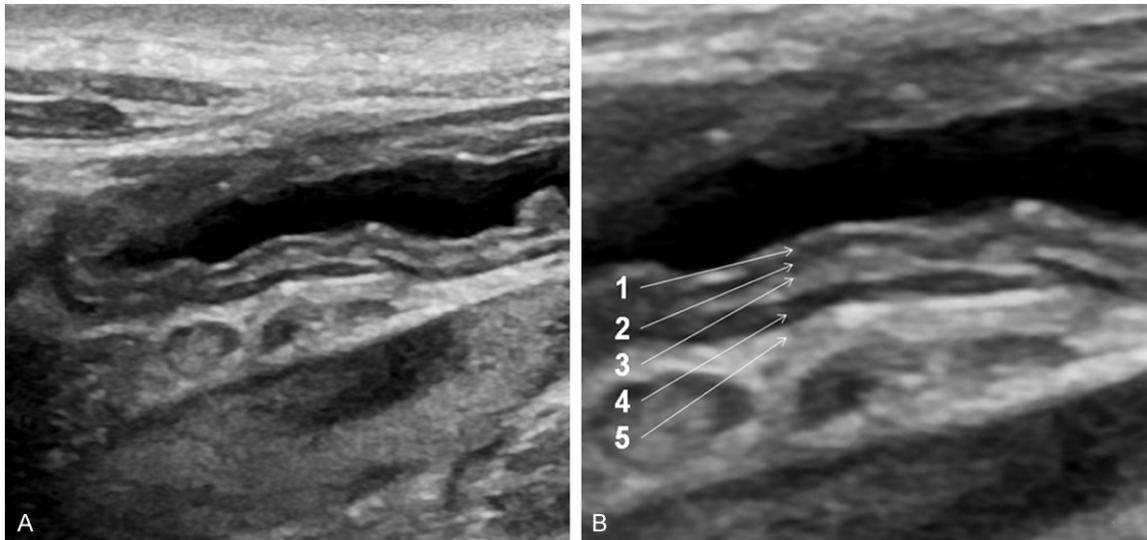
determination of Crohn's disease. However, endoscopy cannot evaluate the intestine proximal to the terminal ileum thus a number of patients were excluded for endoscopy [5]. Endoscopy is an invasive method that is not always well tolerated by patients. In addition, endoscopy cannot provide accurate evaluation of transmural inflammation by Crohn's disease, which can be performed using cross-sectional imaging techniques such as ultrasound, CT and MRI [4].

The European consensus and guidelines have recommended that ultrasound, CT, and MRI are

first choice examinations for the assessment of small bowel lesions in Crohn's disease. CT and MRI are considered to be more accurate and objective in comparison with CDAI score and are equal methods in comparison with endoscopy in evaluating the inflammatory activity of Crohn's disease [6-10]. However, CT and MRI also have disadvantages in the assessment of the disease. First, both scanning methods require intake of contrast agent or colonic luminal distension or bowel cleansing, which means a long-time preparation and discomfort. Second, during CT examination, patients are exposed to a large dose of radiation especially when CT scanning is required in frequent follow-up sessions.

Recently, contrast-enhanced ultrasound (CEUS) after microbubble injection has been proposed as a promising modality of assessing patients with Crohn's disease by determining the enhancement of bowel walls using both qualitative and quantitative methods, with a diagnostic performance comparable with MRI

or CT [11-15]. In addition, CEUS has several advantages over traditional imaging techniques, including lower cost, portability, no radiation exposure, non-invasiveness, and better patient compliance. In comparison with the previous studies that CEUS was mainly used to determine whether the Crohn's disease was in an active period or remission period, the purpose of this study was to discriminate patients with mild active Crohn's disease from those with severe active Crohn's disease using conventional ultrasound, qualitative and quantitative CEUS.



**Figure 2.** Five layers of a normal bowel segment is recognized using conventional ultrasound. From inside outwards, the five layers are: 1, mucosa with hyperechogenicity; 2, muscularis mucosa with hypoechoogenicity; 3, submucosa with hyperechogenicity; 4, muscularis propria with hypoechoogenicity; 5, serosa with hyperechogenicity.

### Materials and methods

#### Patients

The study was approved by the ethics committee of the hospital and informed consent for the study was obtained from all patients as per the World Medical Association Declaration of Helsinki [16].

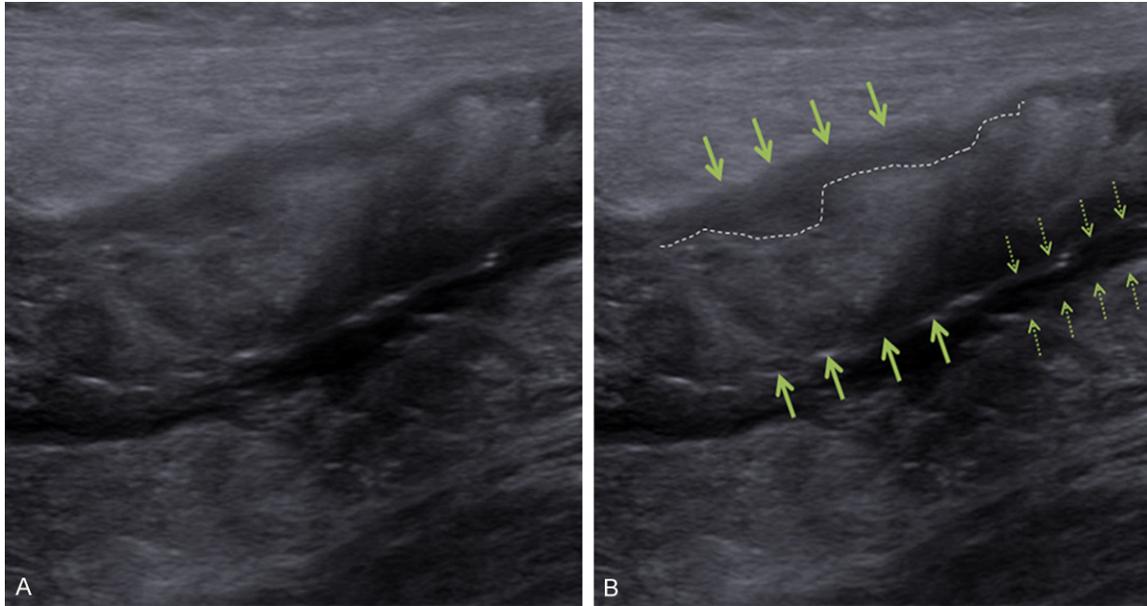
Between August 2010 and May 2013, 50 patients [25 male and 25 female; mean age  $\pm$  standard deviation (SD),  $42.6 \pm 17.8$  years; range 16-82 years] who were hospitalized in the university hospital were recruited into this study. The inclusion criteria were as following: 1. Patients were diagnosed as Crohn's disease according to the diagnostic standard from the Consensus on Treatment Standard of Crohn's disease. 2. A full clinical history of each patient was recorded (**Figure 1**). Thirty-seven (20 male and 17 female; mean age  $\pm$  SD,  $35.3 \pm 13.0$  years; range 18-66 years) of them were considered to be in active period by using both Crohn's Disease Activity Index (CDAI) and endoscopy examination as the reference standard (**Table 1**). The remaining 13 patients were excluded including 10 patients with score 0 on endoscopy and 3 patients with less than 4 mm bowel wall thickness on conventional ultrasound, who were considered to be in the remission period. The time interval between CEUS and endoscopy examination was within one week.

#### Methods

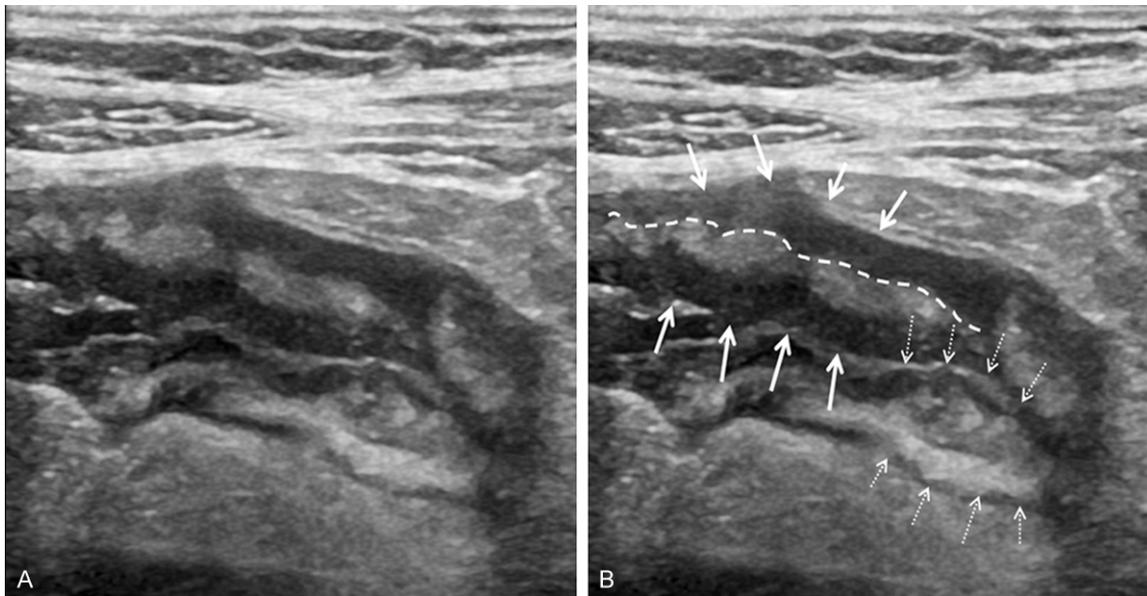
**Endoscopy:** Endoscopy (Olympus, probe CF 260, Japan) of different colonic segments and terminal ileal loops was performed by one experienced gastroenterologist. To reduce intestinal peristalsis and gas inside the lumen, patients were conducted with polyethylene glycol ingestion and overnight fasting, which is necessary for bowel preparation before endoscopy examination.

Mucosal biopsies from different colonic segments and the terminal ileal loop were performed for all patients. After biopsies, the severity of activity of Crohn's disease was evaluated according to the endoscopic Rutgeerts's modified grading system [17]: 0, no lesion found in the terminal ileum; 1, equal to or less than five lesions; 2, more than five lesions with normal mucosa between the lesions; 3, ulcers with diffuse inflamed mucosa; 4, diffuse inflammation with already large ulcers, nodules, and/or stricture. All grading jobs were performed by an experienced gastrointestinal specialist. Using the endoscopic grading of the terminal ileal loop, score 0 is considered as remission stage of Crohn's disease; score 1 and score 2 mean mild disease while score 3 and score 4 correspond to severe activity. According to the endoscopy and/or Rutgeerts's scores, patients with active Crohn's disease were divided into

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**Figure 3.** Intestinal walls of ileum and cecum in a patient with severe Crohn's disease (A, B). The front (between the thick arrows) and the rear (between the thin arrows) parts of the bowel wall could be discriminated by the hyperechoic line of the lumen (B). The thickness of the front entire bowel wall is 15.4 mm, with an obvious thickened submucosa layer. The typical five layers cannot be distinguished. Dichotomy can be applied with the dashed line as the borderline (B). The thickness of interior layer is 13 mm while the thickness of exterior layer is 2.4 mm. In particular, the thickness of hyperechoic submucosa reaches up to 10 mm.

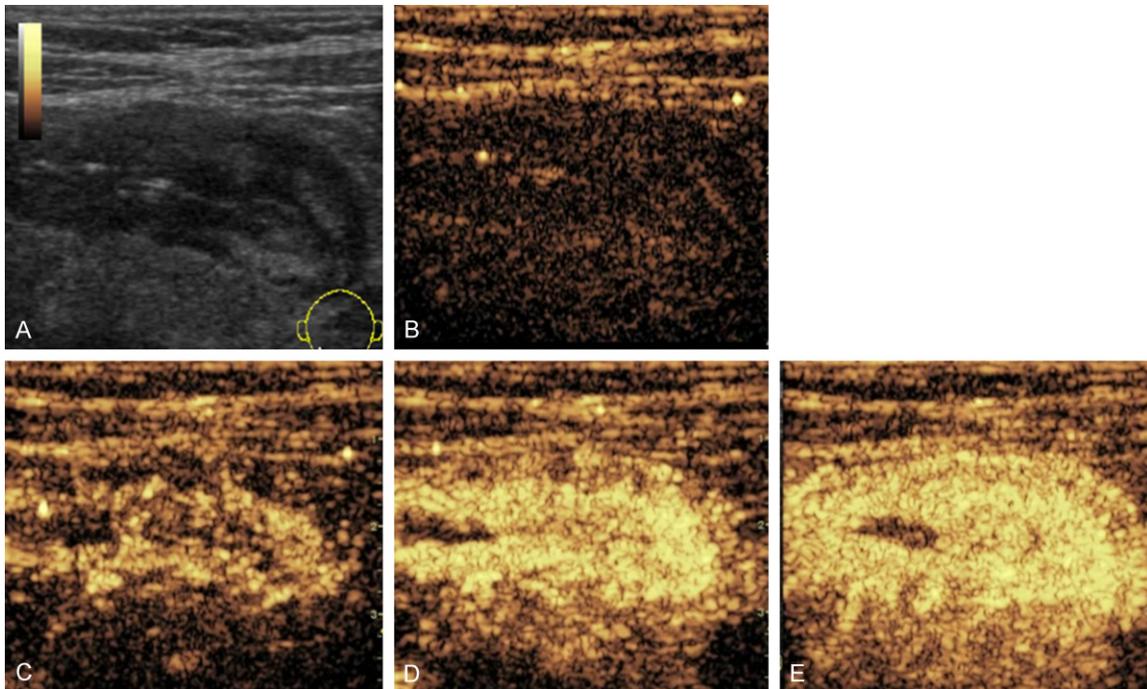


**Figure 4.** Intestinal walls of ascending colon in a patient with mild Crohn's disease (A, B). The thickness is 6.5 mm for the front entire bowel wall (between the thick arrows), 4.5 mm for the interior layer, and 2 mm for the exterior layer. Dichotomy can be applied with the dashed line as the borderline (B). Of the interior layer of bowel wall, the thickness of hypoechoic muscularis mucosa and hyperechoic submucosa is 2.1 mm and 2.3 mm respectively (B). Wall between the thin arrows means the rear part of the bowel wall (B).

two groups: patients with mild disease and those with severe disease.

*Conventional ultrasound:* On the very day of conventional ultrasound examination, all

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**Figure 5.** Conventional ultrasound and CEUS of a patient with severe activity of Crohn's disease. A. Conventional ultrasound shows a thickened ileocecus wall. B. 3 s after contrast agent injection, no enhancement in the wall is detected. C. 12 s after contrast agent injection, punctiform hyperenhancement of the inner layer is observed. D. 16 s after contrast agent injection, transmural hyperenhancement is visualized. E. 19 s after contrast agent injection, transmural equal-enhancement is observed.

patients were required to take 1000-2000 ml of polyethylene glycol in advance. The Logiq E9 ultrasound scanner (GE Healthcare, Milwaukee, WI, USA), equipped with convex array transducer C1-5 (frequency range, 2-5 MHz) and linear array transducer L4-9 (frequency range, 4-9 MHz), was employed in this study.

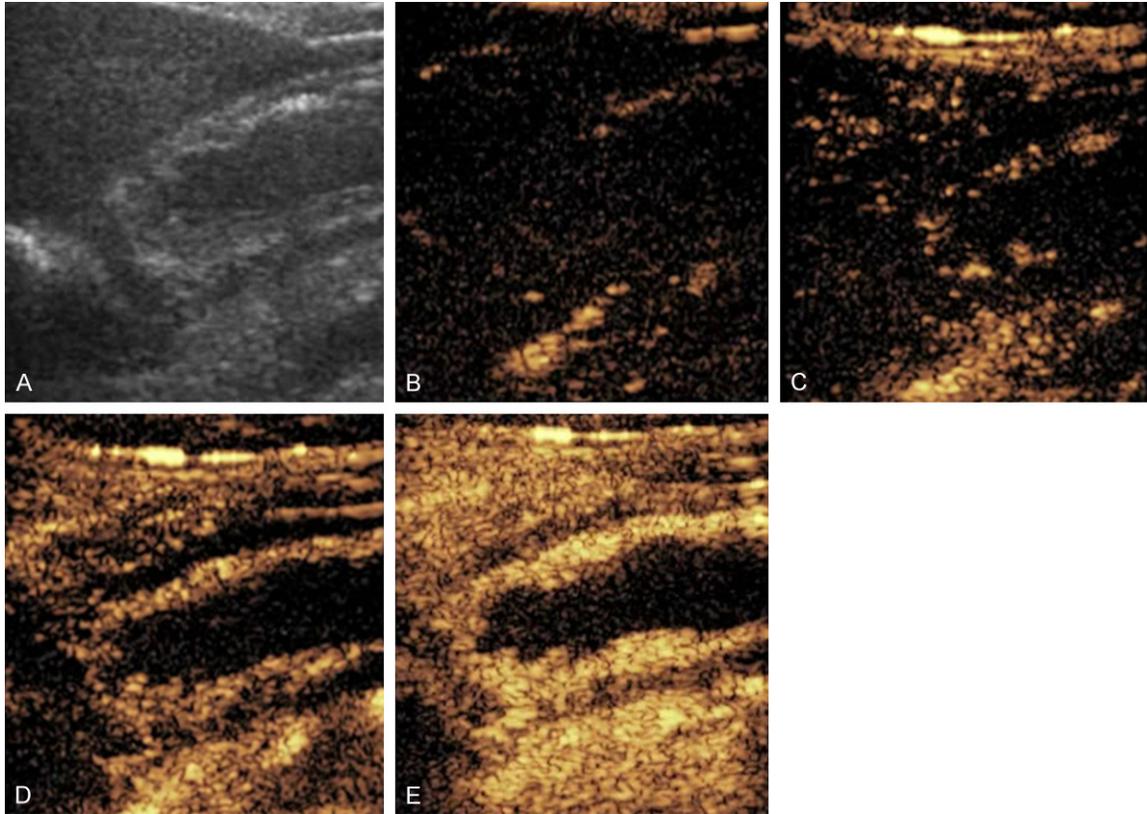
The convex array transducer was used by an experienced radiologist to initially scan the terminal ileums, ileocecus, ascending colons, transverse colons, descending colons, sigmoid colons and small intestinal segments. After that, by utilizing the linear-array high frequency transducer, the examination of possible problematic segments became a priority. During the examination, thickness of intestinal walls, hierarchical structure and echogenicity of the inflammatory regions were evaluated.

Five layers could be recognized by using conventional ultrasound. From inside outwards, the five layers are: 1, mucosa with hyperechogenicity; 2, muscularis mucosa with hypoechoogenicity; 3, submucosa with hyperechogenicity; 4, muscularis propria with hyperechogenicity; 5, serosa with hyperechogenicity (**Figure 2**).

Diagnostic standard of thickened bowel walls was that the thickness of bowel wall  $\geq 4$  mm while they were visible in both longitudinal and transverse scanning. In the present study, the intestinal wall was simply divided into two layers: interior layer and exterior layer, in consideration that in most cases the hierarchical structure of the bowel wall was not clearly visible. The interior layer includes mucosa, muscularis mucosa and submucosa, whereas the exterior layer comprises muscularis propria and serosa.

Bowel segment with the thickest intestinal wall was selected to perform transverse and longitudinal measurement to get the thickness of bowel wall. Interior and exterior bowel wall thickness was also measured and recorded (**Figures 3, 4**).

**CEUS examination:** In each patient, CEUS of the suspicious intestinal wall was then performed after injection of ultrasound contrast agent of sulphur hexafluoride-filled microbubbles (SonoVue, Bracco, Milan, Italy) [18] by one experienced radiologist with 5 years of experience in CEUS. The contrast agent was injected as a



**Figure 6.** Conventional ultrasound and CEUS of a patient with mild activity of Crohn's disease. A. Conventional ultrasound shows a thickened wall of a segment of hepatic flexure of colon. B. 3 s after contrast agent injection, no enhancement in the wall is detected. C. 12 s after contrast agent injection, punctiform hyperenhancement of the inner layer is observed. D. 14 s after contrast agent injection, iso-enhancement is found on the interior layer while no enhancement is observed on the exterior layer. E. 19 s after contrast agent injection, iso-enhancement is found on the interior layer while hypo-enhancement is observed on the exterior layer.

bolus through an antecubital vein at a volume of 2.4 ml, followed by a flush of 5 mL of 0.9 % normal saline solution [19]. A contrast-specific software operating at low acoustic power was applied. The mechanical index settings were adjusted to provide sufficient tissue cancellation with the maintenance of adequate depth penetration. In general, the focus was positioned just below the target intestine segment. The initial gain setting showed system noise in the far field that did not change during the whole examination. Then CEUS mode and a stopwatch were started simultaneously at the time of contrast agent administration. The CEUS process was continuously observed for 180 seconds, neither with any change in the machine settings nor movement of the transducer. Images and consecutive uncompressed Digital Imaging and Communications in Medicine (DICOM) multi-frame cine-clips were acquired for each patient during breath-hold-

ing. All archives were transferred to the picture archiving and communication system (PACS) for further analysis [20-27].

In qualitative analysis, all the thirty-seven patients were included. Enhancement of the intestinal wall in patients with active Crohn's disease was divided into 4 patterns: pattern 1, transmural hyper-enhancement (**Figure 5**); pattern 2, hyperenhancing inner bowel layers and iso-enhancing outer bowel layers; pattern 3, iso-enhancement of both inner and outer layers; pattern 4, iso-enhancing inner layers and hypo-enhancing outer layers (**Figure 6**).

CEUS quantitative analysis was carried out using the SonoLiver<sup>®</sup> software (version 1.0; TomTec Imaging Systems, Munich, Germany). The software was designed for real-time evaluation of tissue perfusion through CEUS examination. To perform quantitative analysis, four

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**Table 2.** Bowel wall thickness of entire wall, exterior and interior layers in 37 patients with different severity of Crohn's diseases

Bowel wall thickness	Mild Crohn's disease (n = 19)	Severe Crohn's disease (n = 18)	P
Entire wall (mm)	6.9 ± 1.9 (3.6-11.0)	9.9 ± 3.5 (7.0-22.4)	0.02*
Exterior layer (mm)	2.4 ± 0.6 (1.0-3.5)	3.0 ± 1.4 (0.8-6.0)	0.06
Interior layer (mm)	4.7 ± 1.7 (3.0-9.0)	7.1 ± 3.1 (4.5-17.4)	0.49

\*Indicates the difference is statistically significant.

**Table 3.** Four enhancement patterns in CEUS qualitative analysis

Endoscopy	CEUS			
	Pattern 1	Pattern 2	Pattern 3	Pattern 4
Mild Crohn's disease (n = 19)	5	3	1	10
Severe Crohn's disease (n = 18)	10	8	0	0

parameters as following were assessed: maximum of intensity (Imax), the percentage ratio of intensity of region of interest (ROI) in lesions and ROI in reference at the highest of the perfusion process; rise time (RT), the time from 10% Imax to 90% Imax; time to peak (TTP), time from contrast arrival to the peak enhancement intensity; mean transit time (mTT), time from appearance of contrast agent to 50% contrast agent washout. Quality of fit (QoF), which indicates the fitness between the raw data and the theoretical curve, is required to be greater than 75% when performing quantification analysis.

**Statistical analysis:** Statistical analysis was performed with statistical software SPSS (Version 18.0; SPSS Inc., Chicago, IL). The quantitative data were expressed as mean ± SD. The comparison of difference for quantitative data between groups was done by t test while the difference for qualitative data between groups was compared with chi-square analysis. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of conventional ultrasound and CEUS in discriminating severe activity of Crohn's disease from mild activity of Crohn's disease. Areas under the ROC curve (AUROC) and the 95% confidence intervals (CIs) of the Az values were calculated. The ROC curve represents sensitivity versus 1-specificity for all possible cut-off values for prediction of severe activity of Crohn's disease. Cut-off values for bowel wall thickness and quantitative CEUS results for the diagnosis of severe activity Crohn's disease were defined using Youden's index. A value of P < 0.05 was considered statistically significant.

## Results

Endoscopy grading system identified 19 patients with mild disease (10 males, 8 females) and 18 patients with severe disease (9 males, 9 females).

### Conventional ultrasound

Differences between the mild active Crohn's disease group and severe active Crohn's disease group were statistically significant in the thickness of entire bowel wall and the thickness of

interior layer of bowel wall, while no difference was found in exterior bowel wall thickness (**Table 2; Figures 3, 4**). In discriminating severe activity of Crohn's disease from mild activity of Crohn's disease, the cut-off value for the thickness of the entire bowel wall was 6.8 mm by ROC analysis, with the AUROC of 0.84 (95% CI: 0.71-0.97), sensitivity of 94.4%, specificity of 68.4%, positive predictive value (PPV) of 61.1%, negative predictive value (NPV) of 69.2%, and Youden's index of 0.628; and the cut-off value for the thickness of the interior intestinal layer was 4.8 mm, with the AUROC of 0.81 (95% CI: 0.67-0.96), sensitivity of 88.9%, specificity of 63.2%, PPV of 85.7%, NPV of 69.6%, and Youden's index of 0.521. The diagnostic performance in terms of Youden's index was slightly higher for entire wall thickness in comparison with interior layer thickness.

### CEUS qualitative analysis

In qualitative analysis, all the thirty-seven patients were included. Enhancement of the bowel wall in patients with active Crohn's disease was divided into 4 patterns (**Table 3**) (**Figures 5, 6**). When pattern 1 and pattern 2 on qualitative CEUS were considered as severe activity whereas pattern 3 and pattern 4 were considered as mild activity, the sensitivity, specificity, PPV, NPV, accuracy, and Youden's index of CEUS qualitative analysis in discriminating severe activity from mild activity were 100% (18/18), 57.9% (11/19), 64.3% (18/26), 100% (11/11), 78.4% (29/37), and 0.579, respectively (**Table 3**).

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**Table 4.** CEUS quantitative analysis results

	Mild Crohn's disease (n = 6)	Severe Crohn's disease (n = 9)	P
<b>Imax</b>			
Entire wall	2106 ± 660 (1331-2928)	9864 ± 6994 (3785-26450)	0.03*
Exterior layer	2311 ± 1360 (739-4672)	7785 ± 6376 (3589-23159)	0.06
Interior layer	2746 ± 911 (1577-3835)	12814 ± 9802 (3377-29492)	0.02*
<b>RT</b>			
Entire wall	19.3 ± 4.1 (15.5-25.9)	21.4 ± 6.0 (12.2-29.8)	0.43
Exterior layer	18.9 ± 2.7 (15.2-22.0)	21.7 ± 7.5 (11.7-36.0)	0.39
Interior layer	19.9 ± 5.6 (14.6-28.8)	19.7 ± 6.6 (8.4-28.0)	0.97
<b>TTP</b>			
Entire wall	23.4 ± 5.1 (18.9-31.8)	28.6 ± 7.3 (17.7-38.3)	0.16
Exterior layer	22.9 ± 3.9 (17.8-28.7)	29.9 ± 8.2 (16.8-40.7)	0.08
Interior layer	24.1 ± 6.8 (17.9-35.4)	26.9 ± 6.2 (17.5-35.4)	0.41
<b>MTT</b>			
Entire wall	51.3 ± 11.3 (41.1-67.2)	52.6 ± 16.4 (25.9-76.4)	0.87
Exterior layer	50.5 ± 6.9 (43.7-62.8)	58.1 ± 30.2 (25.6-129.2)	0.56
Interior layer	53.5 ± 14.6 (35.8-74.4)	51.4 ± 17.3 (25.6-72.7)	0.87

Imax = Maximum Intensity, RT = Rise Time, TTP = Time To Peak, MTT = Mean Transit Time. \*Indicates the difference is statistically significant.

### CEUS quantitative analysis

Fifteen patients (7 male and 8 female; mean age ± SD = 36.40 + 15.58 years, range 19-66 years) were included in the quantitative analysis while the rest were excluded due to evident peristalsis-related movements of small bowels. Correlations between the four parameters (Imax, RT, TTP, and mTT) and severity of Crohn's activity were assessed (Table 4; Figure 7). Quantitative comparison revealed that patients with mild disease and those with severe disease differed only in Imax of inner bowel wall enhancement (2746.9 ± 911 vs. 12814.5 ± 9802.4; P = 0.02) and Imax of entire wall enhancement (2106 ± 660 vs. 9864 ± 6994; P = 0.03), whereas not in RT, TTP and mTT (all P > 0.05).

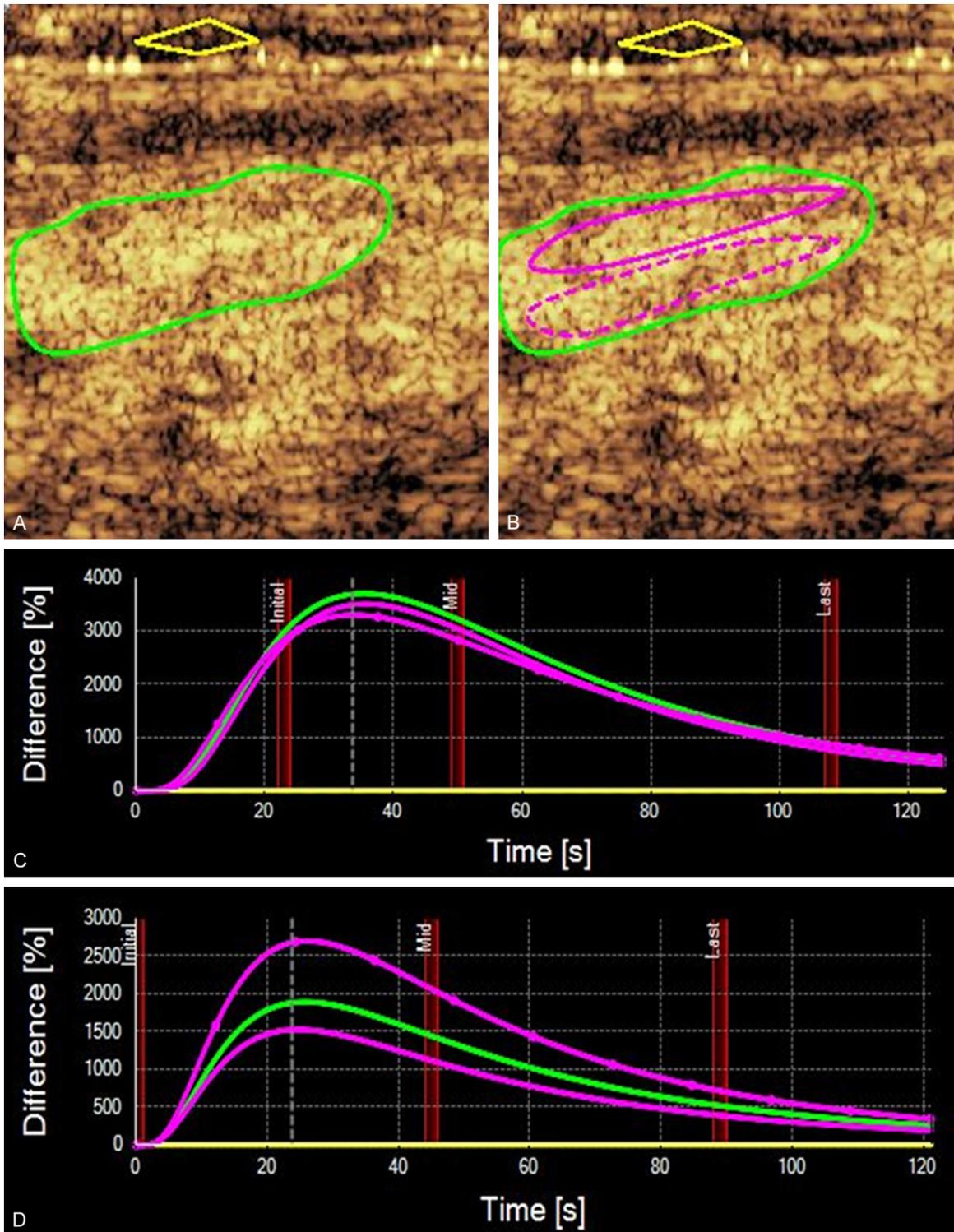
In discriminating severe activity of CD from mild activity of CD, the cut-off value for the Imax of the entire bowel wall was 3067 by ROC analysis, with the AUROC of 0.96 (95% CI: 0.0-1.0), sensitivity of 100 %, specificity of 67.7%, PPV of 100%, NPV of 88.9%, and Youden's index of 0.677; and the cut-off value for the Imax of the interior intestinal layer was 3356, with the AUROC of 1.00 (95% CI: 0.0-1.0), sensitivity of 100%, specificity of 100%, PPV of 100%, NPV of 100%, and Youden's index of 1.0. Therefore, the diagnostic performance in terms of Yo-

uden's index was highest for the Imax of the interior layer, in comparison with all other features on conventional ultrasound, qualitative CEUS, and quantitative CEUS.

### Discussion

As patients with Crohn's disease always suffer a relapse and unexpected complications, frequent examinations will be inevitable. Conventional ultrasound and CEUS can be used as an ideal imaging method to evaluate treatment response and for surveillance because there is no time-interval limitation between the examinations, most importantly, it's radiation-free. Conventional ultrasound was used to detect bowel wall thickness while CEUS was performed to observe vascularization in the bowel wall.

In patients with Crohn's disease, five layers of the bowel wall could be recognized in some patients by using conventional ultrasound. However, during the active period, because of the inflammatory infiltration, the five layers cannot be identified in many patients. Hence, in this study, the concept of interior and exterior layers of bowel walls was introduced. Interior layer includes mucosa, muscularis mucosa and submucosa. Exterior layer comprises muscularis and serosa. Conventional ultrasound reve-



**Figure 7.** CEUS quantitative analysis in a patient with Crohn's disease. A. In CEUS images, area enclosed by yellow line is taken as reference; the circled green line is the region of interest which includes the entire intestinal wall. B. The region surrounded by solid purple line represents exterior layer of the bowel wall while dotted purple line encloses interior layer. C. The fitting curves of the enhancement reveal hyperenhancement of the interior layer and hypoenhancement of the exterior layer. D. The fitting curves of the enhancement reveal hyperenhancement of the interior layer and hypoenhancement of the exterior layer.

aled that interior layer could directly reflect the severity of disease while exterior layer was less influenced, which is largely attributed to the fact that in active period the submucosa is often thickened and hypervascularized whereas the muscularis and serosa layers are less affected. The results also showed that entire bowel wall thickness correlated with the severity of the disease more closely compared with interior bowel wall thickness, thus the entire wall thickness still can be used as a reliable method to evaluate the severity of Crohn's disease.

In a previous study [20], CEUS enhancement of Crohn's disease was divided into four patterns: pattern 1, transmural hyper-enhancement; pattern 2, hyper-enhancing inner bowel layers; pattern 3, hyper-enhancement of only submucosa; pattern 4, no enhancement. Pattern 3 and 4 are normally found in patients in remission period of Crohn's disease while pattern 1 and 2 are always found in patients in active Crohn's disease. By using this classification, sensitivity in determining active period of Crohn's disease was 93.5% while specificity was 93.7% with endoscopy and biopsy as the gold standard. Based on the concept of interior and exterior layer of intestinal wall in the present study, Crohn's disease in active period can be divided into four patterns as mentioned in the Results section. By using the newly introduced classification, the sensitivity in discriminating mild from severe Crohn's disease was 100% while the specificity was 57.9%, with endoscopy and biopsy as the gold standard. Therefore, the specificity of qualitative analysis was not satisfactory in differentiating mild from severe activity of Crohn's disease. Actually, transmural enhancement was found in most of the patients who had active Crohn's disease. Analysis based on the visual observation was limited mainly because the contrast agent inflow was too rapid to make accurate evaluation [28], which made it difficult to determine enhancement patterns. CEUS qualitative analysis also has some advantages. It can be simply employed and the conclusion can be acquired quickly during the examination. Moreover, irregular intestinal peristalsis won't influence the evaluation.

Quantitative CEUS analysis was introduced recently to overcome the shortcomings of qualitative CEUS [29, 30]. Compared to qualitative analysis, quantitative evaluation is a more pre-

cise and reliable method [29]. Quantitative analysis could reduce the influence such as different experience of radiologist and inter-observer variability [30]. As found in this study, I<sub>max</sub> of inner bowel wall enhancement and I<sub>max</sub> of entire wall enhancement on quantitative CEUS were relevant with the severity of Crohn's disease, instead of other quantitative parameters such as RT, TTP, and mTT. In addition, the diagnostic performance in terms of Youden's index was highest for the I<sub>max</sub> of the interior layer, in comparison with all other features on conventional ultrasound, qualitative CEUS, and quantitative CEUS, which indicated that quantitative CEUS is the most accurate method to make a distinction between severe and mild Crohn's disease.

The major drawback of this study is the insufficiency of sample cases because of strict inclusion criteria. Secondly, the endoscopic Rutgeerts's modified grading system was employed as the gold standard that classified cases according to ulcers while ultrasound examination was based on degree of edema and vascularization of intestinal walls. Thirdly, the process of quantitative analysis was complicated and the results could not be obtained immediately. Fourthly, in quantitative analysis no intestinal peristalsis was allowed, so that some cases could not be included into quantitative analysis due to peristalsis. Currently, the software can only eliminate the impact of regular breathing movements, whereas not for the irregular intestinal peristalsis.

In summary, conventional ultrasound, qualitative and quantitative CEUS are able to determine the severity of active Crohn's disease. Quantitative CEUS in terms of I<sub>max</sub> is the most accurate method to make a distinction between severe and mild Crohn's disease. CEUS can be considered as an imaging technique to evaluate the treatment response to some specific therapy for Crohn's disease and to monitor the therapeutic interventions.

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### Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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