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Bowel Perfusion Measured with Dynamic Contrast-enhanced Ultrasound Predicts Treatment Outcome in Patients with Crohn's Disease

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Background: To improve management of patients with Crohn's disease (CD), objective measurements of the degree of local inflammation in the gastrointestinal wall are needed. Increased microvessel density and perfusion are typical features of acute inflammation and can be estimated with contrast-enhanced ultrasound (CEUS). The aim of the study was to investigate whether CEUS can provide prognostic information about patients treated medically for an acute exacerbation of CD.

Methods: Fourteen patients with CD who received medical treatment for acute exacerbation with systemic steroids or tumor necrosis factor- α inhibitors were prospectively recruited. The patients were examined with clinical scoring, blood tests, and CEUS at time 0, 1, 3, and 12 months after initiation of the treatment. Outcome was treatment efficacy or treatment failure defined as change in medical treatment after 1 month or later. The perfusion analysis was performed with a commercially available software program that analyzes the contrast intensity in a selected area, fits the data to a standardized time-intensity curve, and derives several relative perfusion parameters.

Results: Six of the 14 patients had treatment failure during the study period. There was a significant difference between the groups for peak contrast enhancement ($P = 0.013$), rate of wash-in ($P = 0.020$) and wash-out ($P = 0.008$), and the area under the time-intensity curve in the wash-in phase (0.013) at the examination 1 month after the start of treatment.

Conclusions: Perfusion analysis of the intestinal wall with CEUS 1 month after starting treatment in patients with CD can provide prognostic information regarding treatment efficacy.

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Key Words: perfusion, CEUS, Crohn's disease, quantification, ultrasound

Commonly, acute exacerbation of Crohn's disease (CD) is treated with systemic steroids or tumor necrosis factor- α (TNF- α) inhibitors.¹ Currently, there are no optimal methods for assessing the efficacy of a treatment regime. In daily clinical practice, gastrointestinal (GI) endoscopy is normally used to evaluate disease

activity in the GI-tract, but most of the small bowel, subepithelial structures, or extraintestinal complications cannot be visualized with this technique. There are also other limitations with endoscopy, such as risk of perforation, difficulties to pass stenosis, and patient discomfort. Therefore, cross-sectional imaging modalities such as computed tomography, ultrasonography (US), and magnetic resonance imaging (MRI) have become increasingly important.² Ultrasonography seems to perform equally well as both computed tomography and MRI, e.g., in detection of disease and complications as well as in mapping extent and disease activity.^{3,4} Ultrasonography is more operator- and patient-friendly than other imaging methods because new machines are small, portable, relatively inexpensive, and non-invasive and is thus suitable for bed-side examination.⁵ Consequently, it is a modality well suited for the repeated examinations needed when evaluating treatment effect.

Contrast-enhanced ultrasound (CEUS) can be used for estimation of bowel wall perfusion.^{6–8} This is important because neovascularization in the GI wall is a histological finding in active CD related to the degree of inflammation. Also, the autoregulation of the blood supply in the bowel wall is dysfunctional in these patients.^{9–11}

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| | |
|---------|---------------------------------------|
| PE | Peak Enhancement |
| WiAUC | Wash-in area under the curve |
| RT | Rise time |
| mTT | Mean transit time |
| WiR | Wash-in rate |
| WoAUC | Wash-out area under the curve |
| WiWoAUC | Wash in/wash out area under the curve |
| FT | Fall time |
| Wor | Wash out rate |

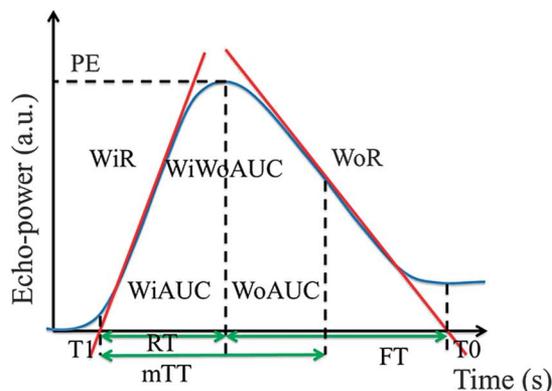


FIGURE 1. Different quantitative perfusion parameters derived from Vuebox. The parameters with their abbreviations are enlisted in the left panel, whereas the figure in the right panel shows how they are derived from the time-intensity curve (the figure is based on a corresponding figure in the Vuebox instruction manual).

There seems to be a correlation between the enhancement from the ultrasound contrast agent, microvessel density,¹² and the histological degree of inflammation.¹³ Several authors have also shown that there is a relationship between contrast enhancement and disease activity in CD both for CEUS and MRI.^{4,14–16} Analyzing the degree of enhancement from ultrasound contrast agents, however, require strict standardization and is thus impractical in daily clinical work. Dynamic contrast-enhanced ultrasound (DCE-US) or analysis of the time evolution of contrast enhancement may be more robust because it is more closely related to the perfusion parameters. Although several studies show that relative perfusion parameters derived from DCE-US are related to disease activity and can separate inflammation and fibrosis,^{7,17–20} few studies have implemented this method in longitudinal studies of treatment outcome.

Already in 2002 using the ultrasound contrast agent Levovist, Di Sabatino et al²¹ found that in a subset of patients with quiescent disease and contrast enhancement, the disease relapsed after 6 months. Recently 2 prospective longitudinal studies were published using endoscopy as reference standard. Moreno et al²² examined patients before treatment and after 1 year with CEUS and found that bowel wall thickness predicted endoscopic remission better than contrast enhancement. Ordas et al²³ examined patients before treatment and after 3 months using an MRI score including contrast enhancement and found a good correlation with endoscopy and an accuracy of 83% for predicting mucosal healing. These studies focus on contrast enhancement not perfusion, but in a recent study by Quiaia et al,²⁴ they found a difference in relative perfusion 3 months after initiation of treatment between treatment responders and nonresponders. This was a cross-sectional study, however, not including data from the start of the treatment or data on bowel wall thickness, which previously has been shown to be related to treatment outcome.^{25,26}

The aim of this pilot study was to evaluate whether perfusion parameters derived from DCE-US of affected bowel with commercially available software can be used for monitoring disease activity in patients with CD. As a secondary aim, we wanted to examine when the most appropriate time to perform the follow-up examination was.

MATERIALS AND METHODS

The study was a prospective pilot study with 12-month follow-up performed in a single center.

Patients

Twenty patients with acute exacerbation of CD, scheduled for treatment with systemic steroids, adalimumab, or infliximab were prospectively recruited from the outpatient clinic or the bedward at the Section of Gastroenterology at Haukeland University Hospital. Inclusion criteria were acute exacerbation

TABLE 1. Different Quantitative Perfusion Parameters with Corresponding Definitions, Derived from the Modeled Time-intensity Curve (TIC) in Vuebox

| Parameter | Abbreviation | Definition |
|---------------------------------------|--------------|--|
| Peak enhancement | PE | Indicates the maximum intensity of the TIC |
| Wash-in area under the curve | WiAUC | Area under the TIC from time of arrival to the PE |
| Rise time | RT | Time from arrival of contrast to PE |
| Mean transit time | MTT | Average time for the blood to pass through the region of interest |
| Wash-in rate | WiR | The maximum slope of the TIC represented as a tangent at the ascending part of the curve |
| Wash-out area under the curve | WoAUC | The area under the TIC from the PE to the end of the curve |
| Wash-in/wash-out area under the curve | WiWoAUC | The total area under the TIC |
| Fall time | FT | Time from PE to a point at the x-axis where the minimum slope tangent crosses |
| Wash-out rate | Wor | The minimum slope of the curve. Represented as a tangent at the descending part of the curve |

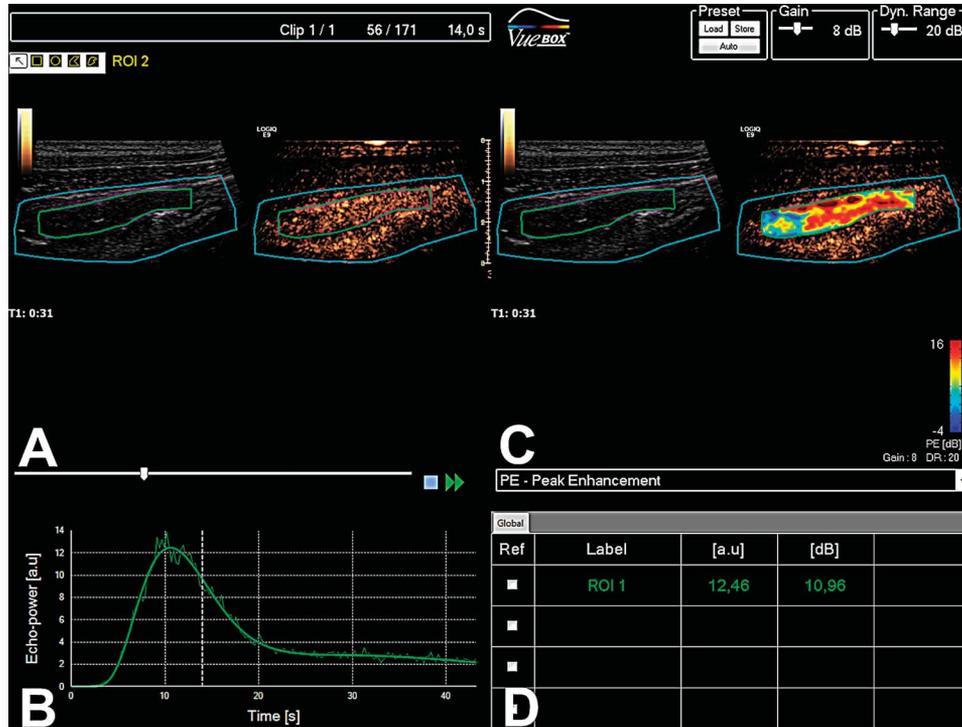


FIGURE 2. CEUS of the intestine in a patient with CD. A, the ultrasound image is presented as B-mode to the left and in a contrast mode to the right. B, Enhancement with Sonovue linearized to arbitrary units together with the modeled time-intensity curve. C, Ultrasound-image with B-mode to the left and a parametric color map covering the contrast image to the right. D, Table displays the quantitative data obtained from the analysis.

of CD with Crohn’s disease activity index (CDAI) >150 requiring medical treatment with either systemic steroids or anti-TNF’s, such as infliximab or adalimumab. Exclusion criteria were normal transabdominal ultrasound, abscess, perforation, or stenosis requiring surgery during the first month of follow-up, pregnancy, age <18 years, acute coronary or pulmonary failure, or allergy against the medical treatment or the ultrasound contrast agent. The patients were recruited from 2009 to 2012. The study was observational and decision-to-treat or change treatment was made by a physician without knowledge of the CEUS findings. Each patient underwent clinical scoring as well as blood and stool sampling at the start of the study and 1, 3, and 12 months into the follow-up period. Ileocolonoscopy was performed before study start to confirm disease status.

Clinical and Biochemical Tests

Patient history, demographic data, and current treatment regime were collected through patient interviews and/or access to medical records. Each patient was scored according to the Montreal classification.²⁷ CDAI was registered before the ultrasound examination. Blood and stool samples were collected within 1 week after the ultrasound examination. Hemoglobin (g/dL), leukocyte count (10⁹/L), platelet count (10⁹/L), C-reactive protein (mg/L), and Albumin (mg/L) were measured in the blood, whereas the stool was analyzed for calprotectin (µg/mg).

TABLE 2. Montreal Classification, Gender, and Outcome for Each Patient with CD Examined in the Study

| Patient | Gender | Montreal Classification | | | Effective Treatment |
|---------|--------|-------------------------|----------|----------|---------------------|
| | | Age | Location | Behavior | |
| 1 | Female | A2 | L2 | B1 | No |
| 2 | Male | A2 | L1 | B2 | Yes |
| 3 | Female | A2 | L3 | B1 | Yes |
| 4 | Male | A2 | L3 | B1p | No |
| 5 | Male | A2 | L3 | B1 | Yes |
| 6 | Male | A3 | L1 | B3 | No |
| 7 | Female | A3 | L2 | B1 | Yes |
| 8 | Male | A2 | L2 | B1 | Yes |
| 9 | Male | A3 | L2 | B1 | Yes |
| 10 | Male | A2 | L3 | B1 | No |
| 11 | Female | A2 | L2 | B2 | No |
| 12 | Female | A3 | L2 | B1 | Yes |
| 13 | Male | A2 | L1 | B2 | No |
| 14 | Male | A2 | L3 | B1 | Yes |

Age of diagnosis: A1 <16 years; A2 = between 17 and 40 years; A3 >40 years; Location: L1 = ileal; L2 = colonic; L3 = ileocolonic; L4 = isolated upper disease; Behavior: B1 = nonstricturing, nonpenetrating; B2 = stricturing; B3 = penetrating; p = perianal disease.

Ultrasound Examination

Ultrasound was performed with a Logiq E9 ultrasound scanner (GE Healthcare, Milwaukee, WI), using a convex probe (C1-5, 1–6 MHz) for abdominal overview, a linear probe (ML6-15, 9–15 MHz) for detailed examination of the intestinal wall and a linear transducer (9L, 5.5–9 MHz) for the CEUS examination. All segments of the small and large bowel were examined. A small and large bowel wall thickness >2 mm was considered pathological if the bowel lumen diameter was >0.5 cm and >3 mm if the lumen diameter was <0.5 cm or collapsed. The length of the affected bowel wall, the bowel wall thickness, and the thickness of the individual ultrasound layers were measured as previously described.^{28,29} The ultrasound layers correspond closely to the mucosa, submucosa, and proper muscle of the GI wall.³⁰ The thickest wall section was chosen for further examination.

DCE-US was performed using Sonovue (Bracco, Milan, Italy) and the contrast mode on the ultrasound scanner. In contrast mode, we used the general setting with color map 2.0, dynamic range 60, MI between 0.09 and 0.12, gain adjusted to reduce background tissue signal, and focus just behind the affected bowel wall. Two contrast injections were performed.

First, the right iliac artery was examined and acted as a reference. The ultrasound probe was positioned over the right iliac artery so that it could be seen at approximately the same depth as the affected bowel and then 0.4 mL of Sonovue was injected over 2 seconds followed by a flush of 10 mL of saline over 4 seconds. For the second injection, 4.4 mL of Sonovue and 10 mL of saline with the same time intervals was given with the imaging plane in the longitudinal direction of the affected anterior bowel wall. This was done 5 to 10 minutes after the first bolus when no contrast signal could be seen in the affected bowel wall. A 60-second cine loop was recorded and exported as Digital Imaging and Communications in Medicine (DICOM) for further analysis.

Quantitative CEUS Analysis

The analysis of the DICOM contrast recordings was performed with dedicated, commercially available software for perfusion analysis (Vuebox; Bracco Suisse SA, Geneva, Switzerland). Vuebox is a quantification software, which evaluates the CEUS data, based on signal intensity changes over time.⁸ The software converts the exported DICOM video data to linear intensity units using conversion algorithms specific for each

TABLE 3. Therapy, Clinical, and Sonographic Features for Each Patient with CD at Each Time Point in the Study

| Patient | Therapy | | | | CDAI | | | | Bowel Wall Thickness, mm | | | | Length of Affection, cm | | | | Affected Bowel Segments | | | |
|---------|---------|--------|-----|------------|------|-----|-----|-----|--------------------------|-----|-----|-----|-------------------------|------|------|------|-------------------------|----------------|----------------|------------|
| | | | | | | | | | Examination Time, mo | | | | | | | | | | | |
| | 0 | 1 | 3 | 12 | 0 | 1 | 3 | 12 | 0 | 1 | 3 | 12 | 0 | 1 | 3 | 12 | 0 | 1 | 3 | 12 |
| 1 | S | S | S | IFX | 246 | 266 | 67 | 38 | 2.4 | 2.8 | 7.4 | 2.0 | 19.5 | 15.3 | 42.9 | 0 | TI, AC, DC, SC | AC, TC, DC, SC | AC, TC, DC, SC | |
| 2 | S | S | ADA | ADA | 235 | 235 | 47 | 26 | 6.8 | 6.8 | 7.6 | 5.2 | 9.9 | 9.9 | 10.8 | 9.3 | TI | TI | TI | TI |
| 3 | ADA | ADA | ADA | ADA | 237 | 57 | 42 | 66 | 5.0 | 1.1 | 3.0 | 1.6 | 15.8 | 17.6 | 31.7 | 0 | AC, TC | AC, T C | AC, TC, DC, RE | |
| 4 | S | S | | IFX | 236 | 135 | 94 | 89 | 2.9 | 3.3 | 3.5 | 1.8 | 5.2 | 4.0 | 10.4 | 0 | TC, RE | TC | TC, SC | |
| 5 | S | S | | | 396 | 151 | 126 | 175 | 2.6 | 3.2 | 4.6 | 6.5 | 11.2 | 5.1 | 7.0 | 10 | TI, DC | TI | TI | TI |
| 6 | IFX | IFX | IFX | IFX | 245 | 67 | 196 | NA | 6.7 | 7.2 | 8.0 | NA | 13.8 | 15.7 | 11.4 | NA | TI | TI | TI | |
| 7 | S | S | S | | 174 | 133 | 180 | 180 | 4.1 | 4.4 | 2.4 | 1.9 | 10.3 | 21.3 | 15.9 | 0 | DC, SC, RE | DC, SC, RE | DC, SC | |
| 8 | S | S | | | 462 | 148 | 76 | 12 | 3.7 | 4.6 | 1.8 | 1.2 | 23.5 | 12.6 | 4.7 | 0 | AC, TC, DC | TC, AC | AC | |
| 9 | S | S | | | 374 | 71 | 0 | 30 | 6.3 | 0.8 | 4.5 | 1.4 | 32.0 | 5.4 | 16.9 | 0 | AC, TC, DC, SC, RE | DC | AC, TC | |
| 10 | S | S | | ADA | 247 | 241 | 235 | 13 | 4.9 | 8.1 | 4.6 | 2.0 | 26.7 | 27.3 | 17.8 | 3.0 | TI, AC, TC, DC, SC | TI, AC, SC | TI, AC, SC | AC |
| 11 | S, ADA | S, ADA | ADA | IFX | 288 | 223 | 217 | 143 | 4.7 | 5.6 | 3.9 | 5.0 | 23.6 | 17.3 | 12.6 | 38.7 | TC, DC, SC | SC | AC, SC | AC, SC, RE |
| 12 | S, IFX | IFX | IFX | IFX | 406 | 134 | 36 | 36 | 3.4 | 4.6 | 4.0 | 1.0 | 23.0 | 17.0 | 15.7 | 0 | AC, TC, DC | AC, TC | AC, TC, SC | |
| 13 | S | S | S | ADA | 160 | 0 | 6.0 | 30 | 6.0 | 5.3 | 6.8 | 7.1 | 12.4 | 17.3 | 11.2 | 12.6 | TI | TI | TI | TI |
| 14 | S, ADA | S, ADA | ADA | ADA | 400 | 95 | 81 | 21 | 4.3 | 2.7 | 1.6 | 2.4 | 13.1 | 13.6 | 6.6 | 7.3 | AC, TI | AC, TI | TI | TI |

For patients that changed drug regime the new therapy is shown in bold. 0 = Before treatment start; 1 = 1 month after treatment; 3 = 3 months after treatment; 12 = 12 months after treatment; S, systemic steroids; Adalimumab, ADA; Infliximab, IFX; NA, not available; I, ileum; TI, terminal ileum; AC, ascending colon; TC, transverse colon; DC, descending colon; SC, sigmoid colon; RE, rectum.

probe, color map, and dynamic range.³¹ The reconstructed echo-power data are directly proportional to the concentration of the microbubbles within the dose range used,³² and as a function of time, these time-intensity signals are fitted to a standardized curve, from which several parameters can be derived (Fig. 1). Vuebox provides parameters related to signal amplitude, time, and a combination of amplitude and time (Table 1) by using wash-in/wash-out kinetics. Because of a proportional relationship between the perfusion parameters derived from Vuebox and regional blood flow, quantitative measurements are possible. The parameters are expressed in arbitrary units, providing perfusion parameters relative to the actual perfusion.⁸ Thus, the clinician is only able to assess whether an area of interest is hyper-, hypo-, or isoperfused in relation to the surrounding tissue. By normalizing the affected area to an internal reference such as the right iliac artery, perfusion parameters from different examinations can be compared.

The workflow in the software consists of uploading the DICOM file, choosing the relevant probe calibration, deleting off plane frames, and choosing an area of interest that is automatically motion corrected. Then the examiner draws a region of interest and marks the time of contrast arrival before the software analyses the time-intensity data (Fig. 2).⁸ The resulting amplitude-related parameters from the examined bowel wall were exported in an excel sheet and normalized by dividing the perfusion parameters expressed in arbitrary units with the corresponding parameters from the right iliac artery. The time-related parameters are independent of contrast concentration making normalization unnecessary.

Outcomes

The endpoints were clinical remission or treatment failure during the follow-up period. Clinical remission was defined as CDAI <150 after 12 months of treatment start. Treatment failure was defined as a change in the drug regime >1 month after treatment start from systemic steroids to anti-TNF or from one anti-TNF to another during the follow-up period. Effective treatment was defined as continued use of same anti-TNF or discontinuation of systemic steroids during the study period. If a patient had to change treatment due to drug's side effects, this was not considered a treatment failure. Treatment change was done by a clinician unaware of the perfusion measurements.

Statistics

The data were analyzed using descriptive statistics and presented with median, minimum, and maximum values. Further comparison of continuous data between the groups was performed using the Student's *t* test or the Mann–Whitney U test. The level of significance was $P < 0.05$. The data analysis was performed using IBM SPSS Statistics software (version 20 for Windows; IBM Inc., Armonk, NY).

Ethical Considerations

The study was approved by the Regional Ethical Committee for Medical and Health Research in Western Norway (REK).

Each patient signed informed consent before participating in the study.

RESULTS

During the study period, 2 patients withdrew from the study, 1 was lost to follow-up, 1 was diagnosed with bowel perforation within 1 month after inclusion, 1 required acute surgery due to bowel obstruction during the first month, and in 1 case, the contrast data were incomplete and could not be analyzed. The remaining 14 patients were 5 women and 9 men with a median age of 33 years (range, 20–50 yr). The Montreal classification for each patient is shown in Table 2. At 12 months, 11 patients were in clinical remission, 2 had still active disease and 1 had surgical resection of the affected area. The treatment failed in 6 of 14 patients during the study period. Because 11 of 14 patients were in remission at the end of the study, a statistical comparison was not done between the groups. In Table 3, an overview of the therapy, clinical, and sonographic features for each patient at each time point in the study is shown.

There were no significant differences between the effective treatment group and treatment failure group, in the demographical, biochemical, and clinical data for any of the time points during the study. In Table 4, these data are shown for the examination done at the start of the study. Also, there were no significant differences for the ultrasound measurements of the bowel wall thickness and the length of the affected bowel. However, there were significant differences in the bowel wall layers. The

TABLE 4. Demographical, Biochemical, and Clinical Data of Patients with CD Measured at the Initiation of the Treatment

| Biochemical or Clinical Marker | Effective Treatment | Treatment Failure |
|---------------------------------------|---------------------|-------------------|
| | Median (Range) | Median (Range) |
| Age, yr | 43 (20–49) | 32 (22–50) |
| Years of sickness ^a | 1 (0.2–8) | 1 (0.1–22) |
| Height, m | 1.7 (1.6–1.86) | 1.7 (1.61–1.88) |
| Weight, kg | 62 (53–90) | 65 (44–101) |
| Hemoglobin, g/dL | 14.0 (11.7–16.2) | 12.7 (11.6–14.9) |
| Erythrocyte volume fraction | 0.39 (0.32–0.45) | 0.38 (0.37–0.41) |
| Leukocyte count, 10 ⁹ /L | 10.2 (7.4–14.3) | 10.1 (6.3–12.9) |
| Platelet count, 10 ⁹ /L | 375 (227–844) | 457 (167–640) |
| C-reactive protein, mg/L ^a | 21 (8–54) | 9 (4–171) |
| Albumin, mg/L | 40 (31–46) | 41 (33–44) |
| Calprotectin, μg/mg ^a | 181 (32–531) | 220 (46–1279) |
| CDAI | 374 (174–462) | 246 (160–288) |

There were no statistical differences between the groups (Student's *t* test/Mann–Whitney U test).

^aMann–Whitney U test.

CDAI, Crohn's disease activity index.

proper muscle layer was significantly thicker after 1 month and the submucosa layer was significantly thicker after 3 months in the group with ineffective treatment (Fig. 3).

Finally, there were no significant differences in perfusion parameters at time 0 and at 3 and 12 months. However, 1 month after the initiation of the treatment, there was a significant difference between the 2 groups for the amplitude-based parameters' peak enhancement ($P = 0.013$), wash-in area under the curve ($P = 0.013$), wash-in rate ($P = 0.020$), and wash-out rate ($P = 0.008$) (Fig. 4). The results for the 2 remaining amplitude-based parameters' wash-out area under the curve ($P = 0.142$) and wash-in/wash-out area under the curve ($P = 0.059$) were not significant. There was no significant difference at any point in the time-related variables (Fig. 5) between the groups.

DISCUSSION

We found that patients with treatment failure after acute exacerbation of CD had increased relative perfusion and a thickened

proper muscle layer compared with those with effective treatment 1 month after the start of treatment. The examination at this time was performed before any change in therapy and could thus predict the treatment outcome. To our knowledge, this principal finding with an early (1 mo) evaluation of treatment effect has not been presented earlier, although Quaia et al²⁴ found a significant difference between responders and nonresponders 3 months after the start of treatment during a follow-up study.

Our study indicates that patients who have an early therapeutic effect have lower local perfusion, which might separate them from patients with poor or no effect. Thus, perfusion evaluated with CEUS may be helpful in follow-up and treatment of patients with CD. Current research implies that angiogenesis correlate with disease activity.^{9,33} Consequently, quantitative assessments of contrast-enhanced ultrasonographic sequences seem to provide a deeper understanding of the pathophysiology in IBD.

It has previously been shown that contrast enhancement with CEUS corresponds well with endoscopy³⁴ and CDAI¹⁴ and

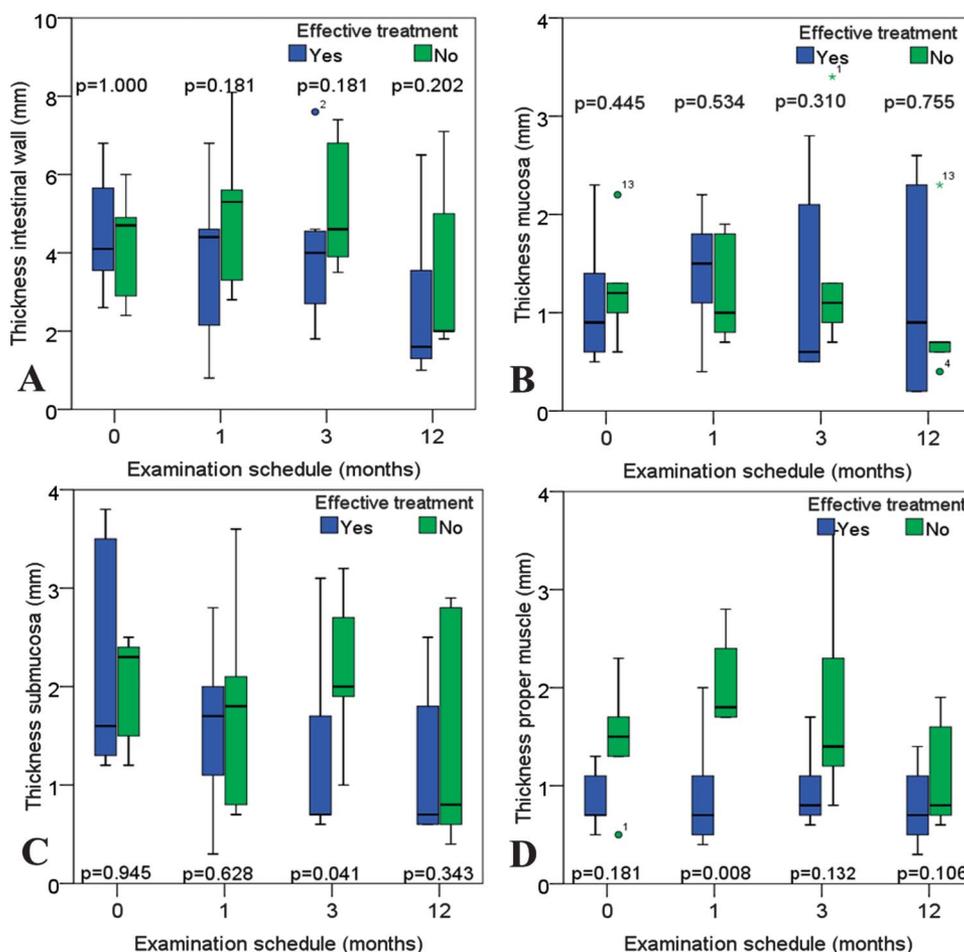


FIGURE 3. Thickness of the intestinal wall and wall layers during follow-up examinations. The box plots colored in green represents the patients with treatment failure, whereas patients with effective treatment are represented in box plots colored in blue. A, Displays the bowel wall. B–D, Presents the thickness of the wall layers mucosa, submucosa, and proper muscle, respectively.

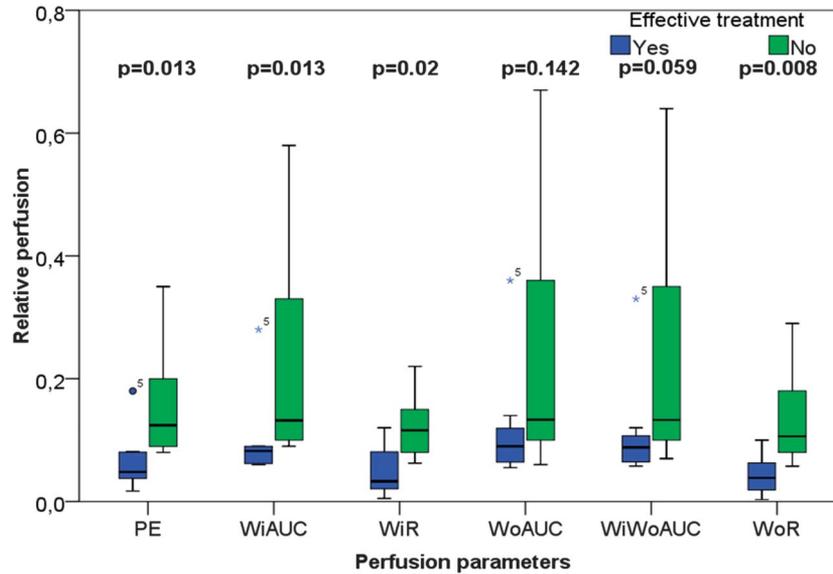


FIGURE 4. Box-plot representation of the contrast analysis related to signal intensities in patients with active CD 1 month after initiating medical treatment. Group 1 (blue boxes) responded well to the treatment, whereas group 2 (green boxes) had treatment failure. The figure displays significant differences (Mann–Whitney U test) between the groups for the perfusion parameters peak enhancement, wash-in area under the curve, wash-in rate and wash-out rate.

can predict disease relapse in CD.³⁵ The degree of contrast enhancement is however dependent on several other confounding factors, such as contrast method and equipment used, depth of lesion and shadowing from air, or arteries filled with contrast.⁶ This can partly be solved by scaling the data, but although it

relates to microvessel density, it does not describe the dynamic element of perfusion since the transit of blood through the tissue is not recorded. DCE-US includes time-intensity data and could thus tell us more of the pathophysiology.

Quaia et al used a scaled time-intensity analysis and found that there was a significant difference in the area under the curve between treatment responders (fall in CDAI ≥ 70 points) and nonresponders after 3 months. The study cannot be directly compared with ours due to a different outcome, but the results do partly correspond. There was, however, no difference in peak enhancement, and data on enhancement rate were not shown. This study was performed on video data that are a log-compressed version of the actual ultrasound intensities, which is mathematically incorrect.³⁶ In our study, the linear data were reconstructed from the video, which may be the reason why we found a significant difference for the peak enhancement and wash-in and wash-out rates.

We found that some amplitude-related perfusion parameters 1 month after treatment start were not significantly different, but there seems to be a group effect. As a limited amount of patients were included, it is possible that the nonsignificant results are due to type 2 errors. Regarding the perfusion parameter wash-out area under the curve, it is possible that this was not significantly different between the groups because of another aspect of the study design. In our study, all CEUS recordings lasted 60 seconds, which could be too short for evaluating wash-out of contrast agents. When assessing wash-in/wash-out area under the curve, a similar argument could be used because the parameter is influenced by the wash-out area under the curve (Table 1). Longer time recordings may consequently be necessary in future studies.

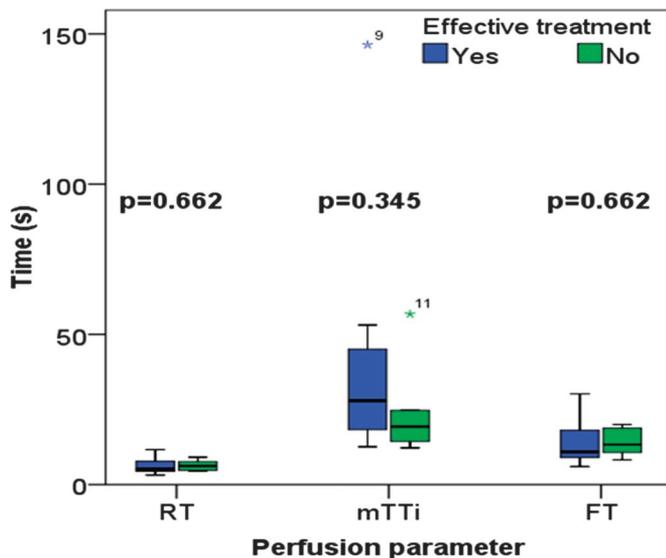


FIGURE 5. Box-plot representation of the results from the time-related variables such as rise time, fall time, and mean transit time in patients with active CD 1 month after initiating medical treatment. Group 1 (blue boxes) responded well to the treatment, whereas group 2 (green boxes) had treatment failure. There were no significant differences between the groups (Mann–Whitney U test).

The time-related perfusion parameters (rise time, fall time, and mean transit time) were not significantly different. This could be due to possible weaknesses in the study design or in the perfusion modeling. When injecting an intravenous bolus and analyzing contrast intensity over time, the bolus profile is dependent in the arterial input function (AIF). The AIF, however, is dependent on injection speed, distance from injection site to observed area, and interindividual differences in the blood vessel system and blood volume. The bolus injection was manual, but standardized. A mechanical injection using an automated pump could reduce the variability in bolus administration, but cannot correct the patient-specific variability. Recently, Jirik et al³⁷ provided a new pharmacokinetic model combining bolus injection and burst replenishment, which could overcome this problem by providing an AIF estimate.

When comparing the thickness of the bowel wall between those with effective and ineffective primary treatment, a significant difference between the groups was expected. In our pilot study, we could not reveal such a difference (Fig. 3). It is reasonable to assume this is also due to a type 2 error. When analyzing the individual bowel wall layers, however, there was a significant difference between thickness of the proper muscle layer after 1 month and in the submucosa layer after 3 months. In CD, thickening of the proper muscle layer is associated with patients with fibrosis in the GI wall,⁷ whereas thickening of the submucosa layer is associated with inflammation.^{7,38} With closer examination of the box plots in Figure 3, one could suspect that the proper muscle layer is thicker in the treatment failure group also before the treatment, although this result is not significant. If these patients already have developed a degree of fibrosis in the GI wall, this may explain why they are less responsive to treatment.

One might argue that measuring the thickness of the proper muscle layer is simpler than estimating perfusion and that this parameter alone seems promising for predicting treatment outcome. Although inflammation is necessary to trigger the process of fibrosis, it seems that it is not necessary for perpetuating it. In fact, once started, the fibrotic process can run without ongoing inflammation.³⁹ It would therefore be reasonable to use markers of both inflammation and fibrosis when evaluating treatment effect in patients. Increased perfusion could indicate ongoing inflammation, whereas thickening of the proper muscle layer could indicate worsening fibrosis.

The major limitation of the study is the small number of included patients. Although the examiner was masked to the patient endpoints, the contrast analysis was performed by the same examiner in all cases. Interobserver agreement should be determined in future studies. Furthermore, all analyses were performed using the same quantification software. In future work, comparison of different perfusion modalities could contribute to increase the reliability of the results. The interindividual differences according to blood flow could not be standardized, which might have influenced the result. Consequently, a study including more patients is required, and future studies should also compare the results according to other outcomes, such as resection, endoscopic remission, and clinical remission.

In conclusion, CEUS enables high-resolution perfusion analysis of the intestinal wall. One month after starting treatment in patients with CD, prognostic information regarding treatment response can be obtained by ultrasound scanning of the bowel.

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