Quantitative contrast-enhanced ultrasound for monitoring vedolizumab therapy in inflammatory bowel disease patients: a pilot study

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Abstract

Background: Microvascularization of the bowel wall can be visualized and quantified non-invasively by software-assisted analysis of derived time-intensity curves.

Purpose: To perform software-based quantification of bowel wall perfusion using quantitative contrast-enhanced ultrasound (CEUS) according to clinical response in patients with inflammatory bowel disease treated with vedolizumab.

Material and Methods: In a prospective study, in 18 out of 34 patients, high-frequency ultrasound of bowel wall thickness using color Doppler flow combined with CEUS was performed at baseline and after 14 weeks of treatment with vedolizumab. Clinical activity scores at week 14 were used to differentiate between responders and non-responders. CEUS parameters were calculated by software analysis of the video loops.

Results: Nine of 18 patients (11 with Crohn's disease and seven with ulcerative colitis) showed response to treatment with vedolizumab. Overall, the responder group showed a significant decrease in the semi-quantitative color Doppler vascularization score. Amplitude-derived CEUS parameters of mural microvascularization such as peak enhancement or wash-in rate decreased in responders, in contrast with non-responders. Time-derived parameters remained stable or increased during treatment in all patients.

Conclusion: Analysis of bowel microvascularization by CEUS shows statistically significant changes in the wash-in-rate related to response of vedolizumab therapy.

Keywords

Crohn's disease, ulcerative colitis, vedolizumab, quantification, contrast-enhanced ultrasound, CEUS

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Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are the main entities of inflammatory bowel disease (IBD) and yield significant morbidity. Recently, Vedolizumab (Entyvio®; Takeda Pharmaceutical, Osaka, Japan), a monoclonal antibody selective for $\alpha 4\beta 7$ integrin, showed therapeutic efficacy in a subgroup of refractory patients, with likely higher efficacy in UC than in CD (1). To monitor disease activity and to determine response to anti-inflammatory therapy, several diagnostic options are available. Besides clinical disease activity assessment, evaluation by endoscopy is the reference that is quite elaborate due to invasiveness, sedation,

pain, and prior necessity for colonic purge. In addition to magnetic resonance imaging (MRI) or computed tomography (CT), B-mode ultrasound measuring bowel wall thickness and using color Doppler flow

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has shown great potential and is used as the primary diagnostic method in daily routine (2).

CD and UC show increased perfusion and blood volume of the bowel wall in cases with high disease activity (3,4). With the aid of dynamic contrast enhanced-ultrasound (CEUS), microvascularization of the bowel wall can be visualized and quantified non-invasively by software-assisted analysis of derived time-intensity curves (5). Analysis of CEUS can be longitudinally compared between different examinations without discomfort (6–8). The intravenously applied ultrasound contrast agent is eliminated via the lungs and undesired side-effects (radiation, kidney failure, and thyroid problems) of contrast-enhanced CT do not exist (9).

In our study, parameters of bowel wall perfusion were evaluated using CEUS to monitor clinical therapeutic outcomes in patients with IBD treated with vedolizumab.

Material and Methods

In this prospective, observational pilot study between August 2014 and May 2015, a total of 34 patients with proven IBD that presented for initiation of vedolizumab therapy (therapy weeks 0, 2, 6, and 14) were screened. This observational study was approved by the Ethics Committee at our institute and written informed consent for CEUS and for the study was obtained from all study patients. Sixteen patients out of the screened 34 patients were excluded from the study due to various reasons such as refusal, interruption of treatment before week 14 or CEUS video slopes of poor technical quality insufficient for software quantification (Fig. 1). In total, 18 patients (11 with CD and seven with UC) completed the study. At baseline (first application of vedolizumab) and after 14 weeks, B-mode ultrasound and CEUS examination were performed.

At the time of each vedolizumab treatment, blood samples (leukocytes, C-reactive-protein [CRP], and hemoglobin) and clinical scoring (Harvey-Bradshaw index [HBI] or clinical Mayo Subscore) were performed. Clinical data and patient characteristics (age, body mass index [BMI], former therapy, and duration of disease) were documented.

Clinical scores

The HBI clinically determined disease activity by five clinical criteria in patients with CD (10). A sum of <5 points indicated clinical remission, 5–7 points mild disease, 8–16 points moderate, and >16 points severe activity. In patients with UC, the clinical Mayo Subscore was determined (11) by three clinical items awarding a maximum of nine points. By means of changes in the clinical score, patients were classified

Fig. 1. Flow chart of screened patients with IBD and final study cohort with Crohn's disease (CD) or ulcerative colitis (UC).

as group 1 "clinical response" (UC: Mayo Subscore $\Delta \leq -1$; CD: HBI $\Delta \leq -4$ or remission at week 14, respectively, and continuation of vedolizumab therapy) or group 2 "no response" (other than group 1).

Ultrasound and quantification

B-mode high-frequency ultrasound with the linear transducer 9L4 and CEUS with the curved array transducer 6C1HD were performed using the Acuson S2000 (Siemens Medical Solution, software version VB21A, Erlangen, Germany). Three unblinded senior physicians with more than ten years of experience performed high-frequency ultrasound and CEUS in the most affected bowel segment. The same location was evaluated in the follow-up. Image size, gain, focus zone, and pulse-repetition frequency were optimized. The affected bowel segment was described regarding bowel wall thickness (mm) and intramural semi-quantitative vascularization grade 0–3 (no color Doppler signal = 0;

few, short signals = 1, long intramural perfusion signals = 2, and intramural and mesentery signals = 3) in a modified version reported by Limberg et al. (4) (Fig. 2).

CEUS was performed with the same settings before and during therapy: low mechanical index (0.04); lowest penetration depth; scale D; frequency of 1.5 MHz; time-gain compensation in middle position; and a dual window. An intravenous injection of a bolus of 2.4-mL ultrasound contrast agent (SonoVue®, Bracco S.P.A., Milan, Italy) was administered followed by a 10-mL flush of saline solution. The linear raw data of the examinations were recorded continuously over a period of 2 min with a frame rate of 16 Hz and exported as DICOM format to an external program called VueBox® (Bracco Suisse SA, Version 4.2.3.0, Software Applications, Geneva, Switzerland). A region of interest (ROI) was defined manually to

the bowel wall transversally in a longitudinal section (Fig. 3). Gas reflections or further artifacts were avoided by the shape of the ROI or by editing the clip.

The time-intensity curves generated by VueBox® were automatically analyzed to calculate CEUS parameters of bolus kinetics. These included amplitude-derived parameters measured in arbitrary units (a.u.) as well as the time-derived parameters measured in seconds (Table 1). The wash-in rate/wash-out rate (WiR/WoR) is the maximum/minimum slope of the time-intensity curve represented as a tangent at the ascending/descending part of the curve.

Statistical analysis

All data were expressed as the mean $\pm$ standard deviation and the range indicated in brackets. Spearman's correlation coefficient (r) was used for analysis of

Fig. 2. (a, b) Patient with CD with improvement of clinical (HBI decreased from 18 to 6 points) and endoscopic activity during treatment: b-mode ultrasound with decrease of bowel wall thickness from 4.4 to 3.5 mm, but persistent color doppler flow.

Fig. 3. (a, b) Same patient with CD seen in Fig. 2: CEUS at baseline and 14 weeks later showing a decrease in peak enhancement (PE) from 181.257 to 42.975 a.u.

Table 1. Overview of amplitude- and time-derived parameters of CEUS quantification.

Amplitude-derived parameters (a.u.)	
PE	Peak enhancement
WiWoAUC	Wash-in and wash-out AUC
WiAUC	Wash-in AUC
WoAUC	Wash-out AUC
WiR	Wash-in rate
WoR	Wash-out rate
Time-derived parameters (s)	
TTP	Time to peak
RT	Rise time
FT	Fall time
mTTI	Mean transit time local

Table 2. Patient characteristics of seven patients with ulcerative colitis (UC) and 11 patients with Crohn's disease (CD).

Patient	UC	CD
Sex (female/male)	1/6	8/3
Mean age (years)	51 ± 9	38.5 ± 13
BMI (kg/m ²)	24.9 ± 4.6	22.7 ± 3.2
Disease duration (years)	7.3 ± 6	13 ± 10
Prior treatment with biologicals (%)	71.4	100
Clinical score	Mayo Subscore	HBI
Baseline	5.1 ± 1.7	11.6 ± 6.5
Week 14	4.0 ± 2.5	9.5 ± 6.4

BMI, body mass index; HBI, Harvey-Bradshaw index.

relationships between variables. For the comparison of related samples, the paired *t* test was performed. In each patient, the percent change from each CEUS parameter measured at baseline and week 14 was calculated and presented related to therapeutic response. Results were illustrated in tables or in a typical boxplot. Small asterisks mark outliers. A *P* value < 0.05 indicated a statistically significant correlation or difference. All reported *P* values were two-sided. Statistical and graphical analyses were performed using the Statistical Package for the Social Sciences (version 19.0.0.1, IBM SPSS statistics, Armonk, NY, USA).

Results

Ulcerative colitis (UC)

All seven patients (6 men, 1 woman; mean age = 51.0 ± 9 years) had sigmoiditis (Table 2). According to the clinical Mayo Subscore, two patients had high inflammatory activity, three had moderate activity, and two had mild disease activity at baseline. Four patients showed a favorable course of disease and formed group 1 including one patient with complete remission. At baseline, patients with UC showed a mean bowel wall thickness of 5.3 ± 0.8 mm (range = 4.1–6.4) and were higher than normal values. Under therapy, bowel wall thickness remained virtually constant with 5.1 ± 1.3 mm at week 14. An increase (of > 1 mm) was found in two patients. An increase of semi-quantitative vascularization of the bowel wall was found in only two patients with no therapeutic response (Table 3).

Crohn's disease (CD)

Eleven patients with CD (3 men, 8 women) had a mean age of 38.5 ± 13 years. All patients received biologic

Table 3. Evolution of bowel wall thickness and semi-quantitative color Doppler vascularization in Crohn's disease (CD) and ulcerative colitis (UC), mean and standard deviation.

Parameter	Responders group 1 (n = 9)		Non-responders group 2 (n = 9)	
	Baseline	Week 14	Baseline	Week 14
Bowel wall thickness (mm)				
All	5.5 ± 0.7	5.1 ± 1.6	6.0 ± 1.1	5.9 ± 1.1
CD	5.3 ± 0.8	5.3 ± 1.8	6.6 ± 0.6	6.1 ± 1.2
UC	5.8 ± 0.7	4.8 ± 1.6	4.7 ± 0.6	5.6 ± 0.7
Vascularization (grade 0–3)				
All	1.9 ± 0.9	0.9 ± 0.8*	1.8 ± 0.7	1.9 ± 0.9
CD	2.4 ± 0.9	1.2 ± 0.8	2.0 ± 0.6	1.5 ± 0.8
UC	1.3 ± 0.5	0.5 ± 0.6	1.3 ± 0.6	2.7 ± 0.6*

**P* < 0.05.

agents in the pre-study history, with 9/11 (82%) receiving two different medical drugs. Four patients had a prior history of surgery (ileocecal resection). At baseline, two patients had severe disease activity, six had moderate activity, and one patient had mild activity. Two patients appeared to be in remission. At week 14, five patients improved with a mean of −8.4 HBI from 14.6 to 6.2 HBI points (two patients of them achieving remission status). One patient remained stable with high activity, and the remaining five patients deteriorated (corresponding to non-responders in group 2). The semi-quantitative color Doppler score was pathologic in all IBD patients at baseline with four patients showing highly elevated hypervascularization (Table 3). At week 14, only one patient remained at grade 3. The decline of vascularization in the responder group failed to be significant (*P* = 0.056).

CEUS results of all patients

At baseline, none of the CEUS parameters correlated with the clinical scores (HBI) in CD, but in UC the clinical Mayo Subscore correlated positively with WiWoAUC, WiAUC, WoAUC, and mTTI. In all 18 patients, bowel wall thickness did not correlate with CEUS parameters, whereas the semi-quantitative vascularization score correlated with some amplitude-derived values such as PE ($r = 0.52$, $P = 0.029$),

WiR, WoR, and WiPI, but not with time-derived CEUS parameters.

An analysis of the intra-individual percent changes of the kinetic parameters of all patients with CD or UC related to therapy response is shown in Table 4. In the responder group, the amplitude-derived parameters such as PE and WiAUC, for example, showed a trend to decrease in all patients and in patients with CD or UC, respectively (Fig. 4). Only the WiR showed

Table 4. Percent change (%) in CEUS parameters between baseline evaluation and after 14 weeks of therapy: responders (group 1) and non-responders (group 2) with Crohn's disease (CD) und ulcerative colitis (UC).

Percent change (%) Parameter	Responders group 1 (n = 9)			Non-responders group 2 (n = 9)		
	All	CD	UC	All	CD	UC
Amplitude-derived (a.u.)						
PE	-40.8 ± 36	-45.3 ± 47	-35.3 ± 21	61.4 ± 189	94.0 ± 228	-2.5 ± 61.2
WiWoAUC	-29.0 ± 45	-46.0 ± 52	-7.8 ± 26	32.0 ± 98	47.9 ± 115	0.3 ± 51
WiAUC	-30.2 ± 47	-43.1 ± 60	-14.1 ± 22	38.1 ± 134	58.2 ± 164	-1.9 ± 40
WoAUC	-28.3 ± 45	-47.5 ± 48	-4.4 ± 32	31.3 ± 88	46.3 ± 102	1.2 ± 57
WiR	$-45.8 \pm 33^*$	$-45.3 \pm 41^*$	$-46.4 \pm 24^*$	125 ± 314	187.8 ± 374	-0.1 ± 95
WoR	-42.7 ± 46	-40.4 ± 54	-45.6 ± 41	170.5 ± 402	251.4 ± 482	8.8 ± 90
Wipi	-40.9 ± 35	-45.7 ± 46	-35.0 ± 19	58.6 ± 181	90.2 ± 217	-4.8 ± 62
Time-derived (s)						
TTP	12.0 ± 39	-0.8 ± 41	28.0 ± 33	24.5 ± 52	16.9 ± 54	39.6 ± 54
mTTI	14.7 ± 23	16.7 ± 30	12.2 ± 16	-10.3 ± 32	-0.2 ± 32	-30.4 ± 26
RT	15.1 ± 41	-3.4 ± 26	38.3 ± 49	18.7 ± 57	20.8 ± 66	14.6 ± 43
FT	28.4 ± 66	-0.9 ± 23	65.0 ± 88	31.5 ± 88	39.2 ± 100	16.0 ± 74

* $P < 0.05$.

Fig. 4. Decrease of CEUS PE in responders and minor changes in non-responders at baseline and week 14 in patients with (a) CD and (b) UC.

a statistically significant decrease in all responders ($P=0.016$) and in group 1 patients with CD and UC ($P=0.038$ and $P=0.037$, respectively). In the non-responder group, the amplitude-derived parameters increased overall and in the CD group. In patients with UC, these parameters tended to remain stable. The time-derived parameters such as TTP, RT, and FT increased, but with no consistent tendency related to response.

Discussion

Our study evaluating CEUS quantification of the bowel wall in patients with CD and UC at baseline and after 14 weeks of treatment with vedolizumab showed a significant change in semi-quantitative color Doppler flow in high-frequency ultrasound and CEUS kinetic parameters. In particular, amplitude-derived CEUS parameters showed a relevant decrease in the response group in patients with CD and UC. The semi-quantitative color Doppler score showed a positive association with amplitude-derived parameters.

B-mode parameters such as bowel wall thickness and color Doppler intensity classification have revealed differences in active and inactive disease that correlated with endoscopic evaluation, but not or weakly with clinical activity scores in CD (12–15). Histopathological inflammation or high vascular density has been found significantly correlated with active disease on endoscopy, baseline ultrasonography, and CEUS with a higher PE and a shorter TTP (16,17).

To our knowledge, this is the first study monitoring therapy with vedolizumab by CEUS quantification; prior studies focused on patients mainly with CD, and rarely with UC, upon treatment with anti-TNF agents. Using a linear transducer, CEUS at baseline and one, three, and 12 months after initiation of CD treatment ($n=14$, thereof six patients with treatment failure) showed a significant difference between responders and non-responders for the amplitude-based parameters (18). Socaciu et al. (19) performed CEUS in CD and UC patients before and after three months of treatment in correlation to endoscopic scores (a ROI in the abdominal muscle served as a reference). Quia et al. (20) defined responders and non-responders 10–12 weeks after initiation of treatment by clinical activity staging and endoscopy in 50 CD patients and performed ileal CEUS quantification at week 6. In 11/15 patients with UC, increasing histopathological inflammation score showed a trend toward higher PE values ($P=0.07$) (21). Overall, these studies showed that amplitude-based parameters (such as PE or AUC) change during therapy, in particular a significant decrease was demonstrated in case of therapy response (proved by clinical scoring or endoscopy). Generally,

the time-related variables as well as bowel wall thickness did not differ significantly between groups at any point. In CD, considering a cut-off of $\leq -52.2\%$ for change in PE, a sensitivity of 77.8% and a specificity of 100% with an AUC of 0.893 were noted (20).

Most of these findings are in accordance with our results: a decrease of amplitude-derived parameters, in particular PE, WiR (statistically significant), and WoR in responders was observed. The changes in CEUS parameters during treatment with vedolizumab tended to be more pronounced in patients with CD than with UC. Wall thickness and time-derived parameters did not show any correlation.

The power of our findings is impaired by certain weaknesses that we share with other authors. In our study, therapeutic response was classified by slight changes in the clinical inflammation scores and not systematically to endoscopic findings that were not available in all patients (18). Thereby studies use different scoring systems for endoscopic or histopathological activity classification and time range of the endoscopic date is variable or not always described (19). Also, the definition of clinical improvement varies between the studies (22) and may be questioned in our cohort. Most patients had a long history of IBD and prior therapy with anti-TNF agents, sometimes with vedolizumab as the final therapeutic option, thus already slight improvements of disease activity were classified as response. The mechanisms of hypervascularization and blood flow are complex and may be variable in our study due to the relatively long course of disease. The patient number is quite small as vedolizumab was recently introduced, and we evaluated both UC and CD. Moreover, a relevant number of patients (47%) had to be excluded, often due to problems with software-assisted analysis of video loops due to gas within the bowel lumen or thin bowel walls, beside interruption of therapy – in other studies exclusion rates have been reported in the range of 23–66% also due to technical problems or loss of follow-up (19,20). In addition, there is no standardized algorithm of analysis. Some studies use a high-frequency linear transducer, whereas we used a convex probe due to its specific potential to detect the microbubbles. The lengths of the video loops may be different. We analyzed bowel wall vascularization directly with one ROI. Others propose a reference ROI in the mesenteric tissue or the iliac artery (18,19). A recent study revealed differences in CEUS parameters between two different high-end ultrasound systems intra-individually in patients with CD (23). Moreover, a certain intra- and inter-observer bias cannot be excluded: the ROI during CEUS may be on a slightly different location than the evaluation of the b-mode parameters. Defining a ROI is very subjective concerning size and shape and may have affected the

calculations. In addition, the definition of contrast arrival time depending on the observer's choice may affect time-dependent parameters. In the current state, software-based analysis of CEUS of the bowel wall seems not to be ready for use in daily clinical routine.

Limitations of our pilot study were the small number of analyzed patients; 47% patients from the screened cohort needed to be excluded. Patients suffered mostly from complex disease (long course of disease, vedolizumab as final therapeutic option). Therapeutic outcome was determined by clinical activity. An intra- and inter-observer bias during high frequency b-mode ultrasound and CEUS analysis may have occurred. Due to these limitations, a direct comparison between study findings might be difficult. Nevertheless, our data are similar to observations found in the literature based on patients treated with anti-TNF agents.

In conclusion, bowel wall perfusion changes under treatment with vedolizumab. A decrease of semi-quantitative color Doppler flow and of amplitude-derived parameters by the sophisticated software-based analysis of CEUS is shown in treatment responders, whereas higher or relatively stable values were found in non-responders.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Goertz, Wildner and Strobel received fees for lecturing tutor in workshops about contrast-enhanced ultrasound (Bracco); Neurath is a consultant (Advisory Boards) for Takeda; Atreya received fees for lectures from Takeda.

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