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Non-Invasive Assessment of Inflammation and Treatment Response in Patients with Crohn's Disease and Ulcerative Colitis using Contrast-Enhanced Ultrasonography Quantification

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ABSTRACT

Background & Aim: Novel biological therapies in Crohn's disease (CD) or Ulcerative colitis (UC) require a proper follow-up for the assessment of bowel inflammation. While endoscopy is the standard method, the imaging techniques using contrast, particularly contrast enhanced ultrasonography (CEUS), are better tolerated by the patients and can be used more frequently. Our aim was to find the usefulness of dynamic CEUS quantification as compared to endoscopy in the assessment of disease activity and in the follow-up under therapy of the patients suffering from either CD or UC.

Method: We have prospectively evaluated 67 patients with UC and 46 with CD, diagnosed by ileo-colonoscopy and biopsy, comparing the endoscopic scores with clinical scores, C reactive protein (CRP), intestinal wall thickness, layer scores after CEUS and TIC parameters (using SonoLiver® software - Imax, RT, TTP, mTT and AUC). For 25 patients with UC and 13 with CD we performed comparisons of the parameters before and after 3 months of treatment and correlated them with the changes in the endoscopic scores.

Results: For UC, time-intensity curves (TIC) volume parameters (AUC) correlated better with endoscopy (ρ=0.64) than the clinical score (ρ =0.62). Other parameters such as CRP and thickness showed significant but less strong correlation, while TIC flow parameters (RT, TTP and mTT) did not show a significant correlation. Results were similar for CD (ρ=0.64 for Imax vs ρ=0.58 for CDAI). The best predictor for endoscopic improvement in both UC and CD was ln(AUC), with a Wilcoxon Z score of 3.76 and 2.61, respectively. There was also a good correlation between the difference of its values and the difference in endoscopic scores before and after the treatment (rho is 0.68 in UC and 0.73 in CD).

Conclusion: CEUS is a useful technique to monitor activity in IBD patiens during therapy.

Key words: IBD – Crohn's disease – ulcerative colitis – CEUS – SonoVue – TIC quantification.

Abbreviations: CD: Crohn's disease; CDAI: Crohn's disease activity index; CDEIS: Crohn's disease endoscopic index of severity; CEUS: Contrast-enhanced ultrasonography; CICDA: Composite index of CD activity; CRP: C-reactive protein; IBD: Inflammatory bowel disease; ROI: Region of interest; RT: Rise time; SES-CD: Simple endoscopic score for Crohn's disease; TIC: Time-intensity curve; TNFα: Tumor necrosis factor α; TTP: Time to peak; UC: Ulcerative colitis.

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD) characterized by alternating episodes of inflammation and remission, as well as by complications such as strictures, fistulae and abscesses, requiring surgical treatment [1]. Novel biological treatments, such as anti-tumor-necrosis-factor-α (TNFα) antibodies, require close monitoring of the evolution of patients by clinical, biological, endoscopic and imaging criteria. Mucosal healing has been proposed to be the goal in such treatments, leading to a decrease in rates of hospitalization and surgery [2-4]. Clinical scoring has shown poor correlation with the mucosal healing [5], while the endoscopic examination is invasive and unpleasant, restricting its repeated use. Therefore, less invasive, objective and reproducible alternative techniques to measure inflammatory activity are required.

Bowel ultrasound with high resolution probes is now considered an alternative imaging technique for the diagnosis and follow-up of patients with IBD, being as accurate as CT and MRI for detecting intramural and extramural extension of

the disease [6, 7]. Contrast-enhanced ultrasound (CEUS) is a new technique consisting of intravenous administration of an ultrasound contrast agent followed by real-time examination, showing the bowel wall microvasculature and vessels from the perienteric tissues. Imaging quantification techniques can estimate perfusion and enable an objective quantitative measurement of the enhancement [8-11].

There are several studies dealing with the assessment of CD activity by CEUS [8, 9, 12-22], mostly comparing the results with either clinical or histopathological scoring (bioptic or after surgery) and considerably fewer concerning the assessment of UC [23, 24]. In most cases, a good to excellent correlation between the CEUS results and disease activity was found.

In our study we aimed to find the usefulness of CEUS quantification parameters, measured by time-intensity-curves (TIC) in the assessment of disease activity (by endoscopic standards) and in the follow-up of treated patients, suffering from either CD or UC. By that, we tried to find early and safe alternative ways to evaluate the response to treatment without subjecting the patients to frequent endoscopies.

MATERIAL AND METHOD

Patients and study design

During a period of 46 months (from June 2009 to April 2013), symptomatic patients with known or suspected CD or UC requiring either an ileo-colonoscopy or flexible sigmoidoscopy were recruited at the Regional Gastroenterology and Hepatology Institute in Cluj-Napoca, Romania. The study was approved by the Ethics Commission of the University of Medicine Cluj-Napoca and the patients gave their written consent.

Each patient in the study was diagnosed according to endoscopy and histopathology findings. The treatment received during the study was not changed between the endoscopical and US examinations, or during follow-up. Both endoscopy and CEUS examinations were performed during the same admission, at no more than two weeks apart. The primary outcome of the study was the correlation between the measured TIC parameters and the endoscopic scores. Secondary outcomes were the assessment of differences in all parameters that appeared at follow-up after three months of treatment and their correlation with the changes in endoscopic scores.

Exclusion criteria from the study were refusal to give consent, difficult patient collaboration or known contraindications for CEUS. Children and pregnant women were also excluded.

Out of a total of 131 patients recruited, 67 were subsequently diagnosed with UC and 46 with CD. All patients underwent the initial US examination, but 2 patients with UC and 4 with CD were excluded because the CEUS analysis was not possible due to out-of-plane movements. The clinical scores were calculated, according to Truelove-Witts and CDAI scores, respectively. Truelove-Witts scores were coded: 0=remission, 1=mild, 2=moderate, 3=severe. CDAI scores were transformed in levels of severity similar to Truelove-Witts and coded: 0=remission (CDAI≤150), 1=mild disease [151-220], 2=moderate disease [221-450) and 3=severe disease (CDAI≥450).

For the assessment of treatment response, patients were asked to repeat all tests (including endoscopy and CEUS) after a period of 3 months, but the sample size shrank to 38 patients (25 with UC and 13 with CD). Due to complications, 1 patient with UC and 10 patients with CD underwent surgery, while 8 patients with UC and 2 patients with CD had to change treatment; 28 patients with UC and 9 patients with CD did not agree to continue the follow-up, and 2 patients with CD died from complications. Finally, CEUS images obtained at follow-up in 3 patients with UC and 4 with CD were unsuitable for quantification.

Endoscopy assessment

For ulcerative colitis, the Mayo score was used to depict the lesion severity: 0=normal, 1=erythema, decreased vascular pattern, mild friability, 2=marked erythema, absent vascular pattern, friability, erosions, 3=ulcerations, spontaneous bleeding [25].

For CD, the simple endoscopic score for Crohn's Disease (SES-CD) quantified luminal lesions for the same bowel segment investigated by CEUS. The parameters used in this score depict the size of ulcers, the ulcerated surface, the affected surface and the presence of strictures. Each parameter is quantified from 0 to 3, and the sum gives the endoscopic score for the involved segment, also investigated by CEUS [26].

Endoscopic improvement was defined in both diseases as a decrease of at least 1 unit or a value of 0 in the corresponding score at the 3 months time point.

Ultrasound examination

Native US and CEUS studies were performed by one experienced radiologist with over 6 years of experience in CEUS. The US examinations were performed in the morning, in patients after overnight fasting. The examiner was blinded for the clinical data concerning the disease activity. The US examinations were performed using a GE Logiq 7 machine with a 1-5 MHz convex and a 3-8 MHz linear transducer. The scanning protocol consisted of an initial systematic survey of the four abdominal quadrants while performing some compression. The most relevant bowel segment (showing changes such as wall thickening, changes in layer structure, fat stranding, wall feature effacement, strictures or Doppler signal increase) was selected for further analysis. In the case of multiple segments with similar involvement, the most accessible one was chosen. Wall thickness was measured in the longitudinal plane from the mucosal inner hyperechoic line to the serosal outer hyperechoic line.

CEUS examination and TIC quantification

The CEUS studies were performed on selected bowel segments, in longitudinal sections with the 1-5 MHz probe, a Mechanical Index of 0.12 and the focus point deeper than the bowel. We injected 2.4 ml of SonoVue® (Bracco) contrast solution i.v. in bolus through a 20G intravenous cannula into an antecubital vein, than flushed it with 10 ml saline. The timer was set at injection and the enhancement was observed in real time and saved on two continuous clips, totaling 60s. We calculated a Layer score with the method proposed by

Serra et al. [21], corresponding to a complete enhancement of the bowel wall (1), enhancement of the inner layers, up to the muscularis propria (2), enhancement of the submucosal layer alone (3) and absence of enhancement (4).

The clips were processed by the same examiner using the commercial product SonoLiver (Bracco, CH), where they were joined and adjusted for in-plane motion, while segments with out-of-plane motion and other technical problems were cut out. The abdominal muscle was chosen as a reference to calculate intensity values. The ROI was drawn as a freeform shape containing as much of the affected anterior wall, from mucosa to serosa. The program delivered TICs as well as TIC parameters: Maximum intensity (Imax), Rise time (RT), Time to peak (TTP) and Mean transit time (mTT). The results of such an analysis, as well as the US images are shown in Fig. 1. The area under the curve (AUC) was calculated after exporting the interpolated curve data from SonoLiver to a data analysis software (OriginPro 9, OriginLab®) as an integral of intensity vales over a period of 50 s from contrast arrival. We also calculated the natural logarithm of AUC – ln(AUC), considering it a better indicator for high variances of AUC (because the intensity values are obtained from raw linear data). For reference concerning TIC parameters, see Table I and Fig. 2.

Table I. The perfusion parameters and their description

Abbreviation	Definition	Unit
Imax	Maximum Intensity (with respect to the Reference ROI)	[%]
RT	Rise time (independent of the time origin)	[s]
TTP	Time To Peak (coresponding to Imax)	[s]
mTT	mean Transit Time corresponding to the center of gravity of the perfusion model	[s]
AUC	Area Under the Curve, calculated as an integral of the intensity curve	[%][s]

Statistical analysis

The statistics, numerical analysis and the plots were done using the OriginPro 9 software (OriginLab®). We used non-parametric tests for continuous parameters that deviated from normality and summarized the values as medians and quartiles. For all the statistical tests we used a significance level α=0.05. We used Spearman's correlation coefficient and its associated test to assess the strength of the relationship between the endoscopic scores - Mayo endoscopic score for UC and Simple Endoscopic Score for Crohn's Disease (SES-CD) for CD - and the clinical scores, CRP, bowel wall thickness, Layer score, Imax, RT, TTP, mTT, AUC and ln(AUC). The distribution of the parameters listed above, corresponding to intervals in the endoscopic scores was plotted as box-charts. To compensate for different scales, values were normalized in the [0,100] range. Independent predictors of endoscopic disease activity were found using Kruskal-Wallis ANOVA. Discriminators between different levels of endoscopy scores were found using the Mann-Whitney test with the Bonferroni correction.

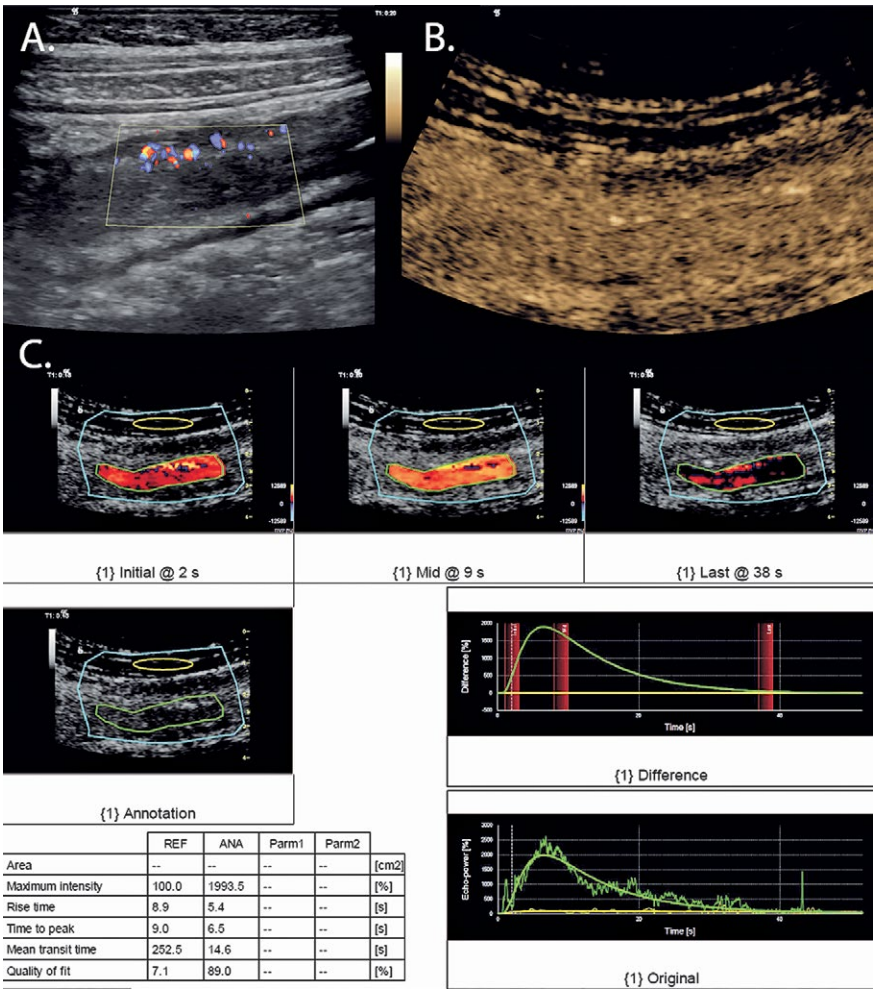

Fig. 1. Bowel segment in a patient with moderate activity Crohn's disease: A. color Doppler and B. arterial phase CEUS showing intense vasculature, especially in mucosal and submucosal layers; C. analysis of signal intensity in SonoLiver® with the position of ROI, the reference ROI in the muscle, color maps at different timeframes, the shape of the TIC and the resulting parameters.

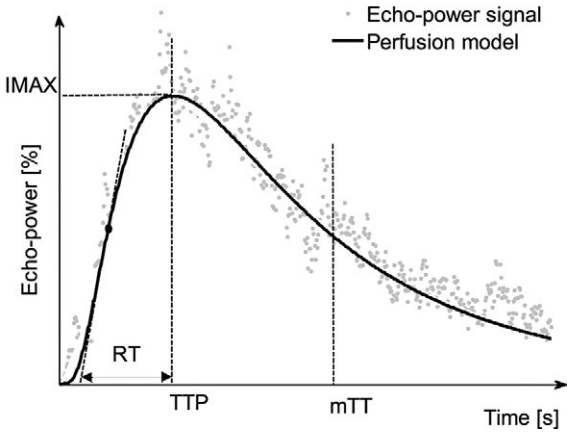

Fig. 2. The perfusion model obtained through fitting of the TIC data by SonoLiver and corresponding perfusion parameters (from SonoLiver user guide)

For the prediction of disease improvement, we tested the significant differences between clinical scores, CRP and CEUS TIC parameters before and after 3 months of treatment using the paired Wilcoxon Signed Rank test for patients with endoscopic score improvement. Spearman's correlation coefficient was used to describe the relation between the differences in endoscopic scores and studied parameters.

RESULTS

The demographics and clinical features of the studied patients are illustrated in Table II.

In two cases of UC and four cases of CD, the CEUS examinations were inappropriate for quantification, with the remaining 65 cases of UC and 42 cases of CD being evaluated in our statistics. Medians, first and third quartiles, as well as Spearman's correlation coefficients between endoscopic scores and studied parameters, with the corresponding tested probabilities for both UC and CD are found in Table III.

Table II. Demographics and clinical features of the recruited patients

Variables		UC	CD
No. of patients (%)		67 (59.3)	46 (40.7)
Age	Median years (Min-Max)	39 (17-72)	37 (18-76)
Gender no. (%)	F	34 (50.7)	22 (47.8)
	M	33 (49.3)	24 (52.2)
Duration of disease	Median years (Min-Max)	4 (1-36)	4 (0.5-20)
Evolutive pattern no. (%)	First flare	20 (29.9)	9 (19.6)
	Chronic continuous	4 (5.9)	11 (23.9)
	Relapsing / remitting	43 (64.2)	26 (56.5)
Montreal classification:			
UC severity no. (%)	Remission (S0)	11 (16.4)	
	Mild (S1)	14 (20.9)	
	Moderate (S2)	20 (29.9)	
	Severe (S3)	22 (32.8)	
UC extent no. (%)	Ulcerative proctitis (E1)	0 (0)	
	Left sided UC (E2)	38 (56.7)	
	Pancolitis (E3)	29 (43.3)	
CD behaviour no. (%)	Non-stricturing, non-penetrating (B1)	30 (65.2)	
	Stricturing (B2)		9 (19.6)
	Penetrating (B3)		7 (15.2)
	+ Perianal disease (p)		4 (8.7)
CD location no. (%)	Ileal (L1)		16 (34.7)
	Colonic (L2)		13 (28.3)
	Ileo-colonic (L3)		17 (37.0)

CD: Crohn's disease; UC: ulcerative colitis

Distribution of the US parameters and CRP values corresponding to Mayo endoscopy scores are plotted as box-charts in Fig. 3. The significant differences, found using the Mann-Whitney test with the Bonferroni correction are shown

Table III. Comparative evaluation of the parameters studied in patients with ulcerative colitis (UC) and Crohn's disease (CD)

Parameters	Ulcerative colitis				Crohn's disease			
	Median	Q1-Q3	Spearman		Median	Q1-Q3	Spearman	
			rho	p			rho	p
Endoscopy score	2	2.00 - 3.00	-	-	5	4.00 - 6.00	-	-
Clinical score	2	1.00 - 3.00	0.62	<0.001	2	1.00 - 2.00	0.58	<0.001
Thickness	6	4.30 - 6.80	0.32	0.010	6.15	5.00 - 7.50	0.39	0.011
Layer score	3	2.00 - 3.00	0.12	0.322	3	2.00 - 3.00	0.45	0.003
CRP	3.22	0.49 - 9.58	0.54	<0.001	1.27	0.48 - 2.53	0.59	0.001
Imax	767.99	387.8 - 1898.6	0.60	<0.001	721.15	328.1 - 1993.5	0.68	<0.001
RT	7.51	5.92 - 9.48	0.05	0.707	6.48	4.57 - 10.16	-0.13	0.401
TTP	9.20	7.37 - 11.90	-0.02	0.904	9.07	5.34 - 12.30	-0.11	0.501
mTT	37.94	20.32 - 80.41	-0.15	0.219	27.07	18.98 - 84.31	-0.18	0.266
AUC	14329	5851 - 41750	0.64	<0.001	13324	5842 - 34005	0.63	<0.001
ln(AUC)	9.57	8.67 - 10.64	0.64	<0.001	9.49	8.67 - 10.43	0.63	<0.001

For abbreviations see text.

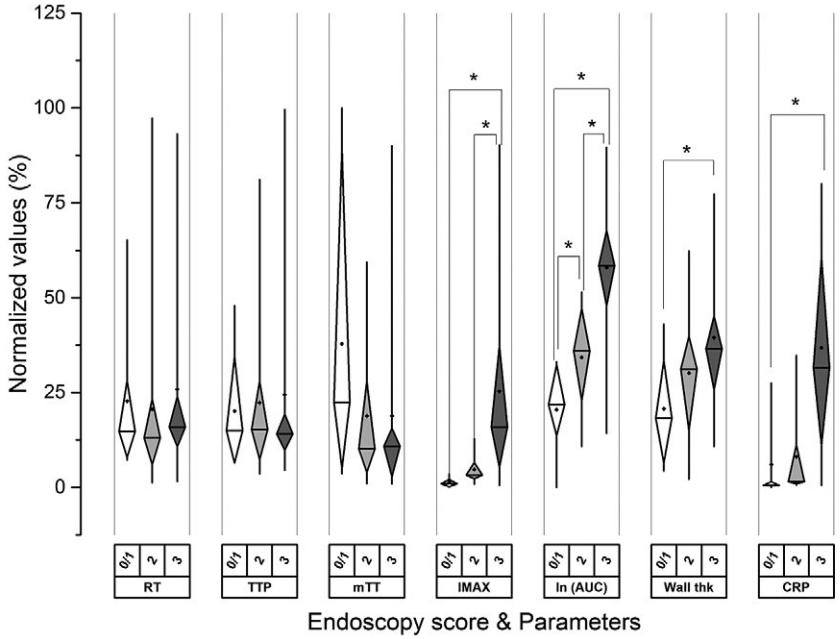

Fig. 3. Box-chart showing the distribution of studied parameters, with values normalized in the [0,100] interval, in correspondence to the Mayo endoscopic score in ulcerative colitis. Statistical significant differences are marked with (*).

with asterisks. Endoscopy scores 0 and 1 have been joined in the same group. Kruskal-Wallis ANOVA test revealed: Truelove-Witts score ($p<0.001$), Imax ($p<0.001$), ln(AUC) ($p<0.001$), Layer score ($p<0.001$), Thickness ($p=0.038$) and CRP as independent predictors for the Mayo endoscopy score ($p=0.001$).

Distribution of the US parameters and CRP values corresponding to SES-CD are plotted as box-charts in Fig. 4. The significant differences, found using the Mann-Whitney test with the Bonferroni correction, are shown with asterisks. For easier reading, SES-CD values were grouped as ≤3, 4-5, 6-7 and ≥8. Kruskal-Wallis ANOVA test revealed only Imax ($p<0.001$) and ln(AUC) ($p<0.001$) as independent predictors for the SES-CD.

The median values with lower and upper quartiles for the tested parameters before and after treatment can be compared for UC in Table IV, and for CD in Table V. For each patient, the differences that occurred in these parameters during treatment have been compared with the differences in the endoscopic score rankings and the correlation coefficient ρ (Spearman rho)

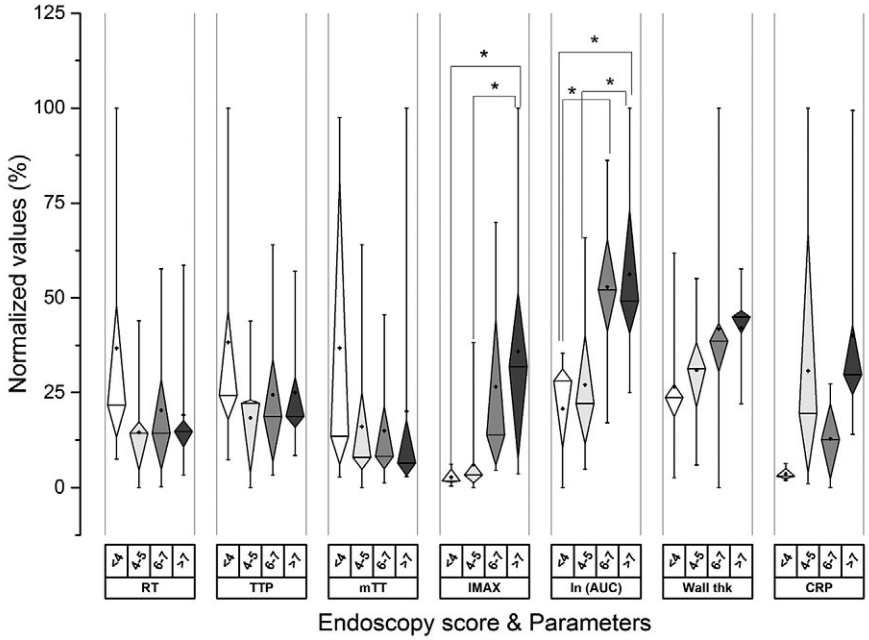

Fig. 4. Box-chart showing the distribution of studied parameters, with values normalized in the [0,100] interval, in correspondence to the SES-CD in Crohn's disease. Statistical significant differences are marked with (*).

Table IV. Changes in the evaluated parameters in the patients with ulcerative colitis (UC) before and after treatment

Parameters	Before treatment	After treatment				
	median (Q1-Q3)	median (Q1-Q3)	Wilcoxon		Spearman	
			Z	p	rho	p
Endoscopy score	3 (2– 3)	2 (1– 2)	-	-	-	-
Clinical score	3 (2– 3)	1 (0 – 1)	2.86	0.004	0.44	0.025
Thickness	5.25 (4.2 – 6.7)	5.1 (4 – 6.8)	0.85	0.395	0.28	0.172
Layer score	2 (1– 2)	3 (3 – 3)	-2.62	0.008	-0.45	0.022
CRP	8.7 (1.91 – 16.25)	0.59 (0.35 – 6.13)	2.64	0.008	0.47	0.029
Imax	1266 (591 – 5389)	351 (193 – 595)	3.56	<0.001	0.62	0.001
RT	7.39 (6.57 – 9.47)	7.51 (5.6– 11.85)	-0.3	0.762	0.03	0.861
TTP	9.12 (8.08 – 10.48)	9.29 (6.73 – 12.97)	-1.67	0.095	-0.27	0.188
mTT	27.3 (15.69– 48.69)	67.21 (23.14 – 107.1)	-1.35	0.177	-0.31	0.132
AUC	32905 (11417 – 70854)	5274 (4103 – 10824)	3.76	<0.001	0.61	0.001
ln(AUC)	10.4 (9.34 – 11.16)	8.57 (8.32 – 9.28)	3.76	<0.001	0.68	<0.001

For abbrevations see text

with the corresponding p significance value also shown in the tables, together with Z scores and p values from the Wilcoxon paired signed-rank test.

DISCUSSION

There is currently no generally accepted method for monitoring the IBD treatment. Traditionally, the bowel inflammation assessment in the follow-up of treated UC or CD is achieved by endoscopy, in the context of symptom worsening. Although in most centers the primary aim is the control of symptomatology, recent studies in patients with CD [2-4, 27] have shown that in many cases where clinical remission has been reached, endoscopic mucosal healing was not achieved. It is not clear though whether stopping treatment in these cases would lead to a faster recurrence [28]. Similar results have been obtained for UC [29]. Moreover, the need for subsequent abdominal surgeries is similarly reduced for patients achieving complete (SES-CD<6) and partial (SAS-CD<6) mucosal healing compared to non-responders [3].

Several studies have evaluated the potential of CEUS in assessing CD activity. Early studies [30, 31] have shown the potential of the method but either lacked a standard for comparison or used only qualitative contrast assessments. Semi-quantitative [19] and quantitative [18] measurements of CEUS enhancement have shown a good correlation with the inflammatory activity as shown by endoscopy. In the study by Serra et al. [21], a semi-quantitative layer score based on enhancement as well as a quantitative E/W ratio based on thickness ratios between the width of enhanced layers and the total wall thickness were proposed, the first score showing a slightly better performance (PPV=63%, NPV=81%) based on clinical remission. As far as we know, only three studies have compared CEUS perfusion with CD activity in endoscopy [13, 18, 22]. In the study by Ripolles et al. [18] performed in 61 patients the only measured CEUS parameter was the relative intensity increase, calculated from TICs produced by the US machine, which showed a significant increase in patients with active vs inactive disease (91% vs 40%). In the study by Wong et al. [13] in 30 patients, TTP, Peak intensity and AUC obtained

Table V. Changes in the evaluated parameters in the patients with Crohn's disease (CD) before and after treatment

Parameters	Before treatment	After treatment	Wilcoxon		Spearman	
	median (Q1-Q3)	median (Q1-Q3)	Z	p	rho	p
Endoscopy score	6 (5– 8)	4 (3– 5)	-	-	-	-
Clinical score	2 (1– 3)	0 (0 – 1)	2.30	0.021	0.51	0.042
Thickness	6,8 (5,8– 7,3)	5,1 (4,45 – 6,75)	2.13	0.033	0.65	0.015
Layer score	2 (1– 3)	3 (3 – 3)	-1.93	0.053	-0.61	0.021
CRP	0,85 (0,525 – 2,45)	0,75 (0,32 – 0,97)	1.35	0.177	-0.14	0.729
Imax	538 (517 – 4159)	308 (172 – 529)	2.61	0.009	0.53	0.039
RT	5,55 (3,575 – 7,25)	8,44 (6,855– 11,65)	-2.37	0.018	-0.59	0.011
TTP	9,18 (5,53 – 10,085)	12,24 (9,445 – 12,63)	-1.77	0.076	-0.48	0.091
mTT	27,3 (17,06– 58,24)	45,95 (28,23– 99,92)	-2.61	0.009	-0.54	0.029
AUC	18806 (8304 – 108865)	6235 (4748 – 10379)	2.61	0.009	0.54	0.029
ln(AUC)	9,84 (9,03 – 11,08)	8,74 (8,47 – 9,24)	2.61	0.009	0.73	0.004

For abbrevations see text

also from TICs produced by the US machine have shown no correlation with the CDEIS values, but TTP significantly decreased after successful treatment. De Franco et al. [22] compared the values obtained in 54 patients, by TIC analysis (maximum peak intensity - MPI, and wash-in slope β) mainly with composite (CICDA) scores, but also secondary with clinical (CDAI) and endoscopic (SES-CD) scores. Significant increases in the TIC parameters (especially MPI) were found in patients with active disease according to all three criteria.

Other authors have compared TIC parameters with clinical [8, 16, 32], postoperative [12, 17] or biopsy [33] histopathology scores. The general trend observed from these studies was the positive correlation between contrast volume parameters (Imax/Peak enhancement, AUC, Regional blood volume) and the activity markers and the negative correlation between contrast flow parameters (TTP, RT, mTT) and the same markers.

There are considerably less studies in the literature concerning the value of CEUS perfusion in assessing UC activity. To the best of our knowledge, there is only one previous study, by Girlich et al. [23] comparing the TIC parameters in 15 patients with histopathological scoring from samples obtained by colonoscopy. They obtained a good correlation but no statistical significance between the Imax and histopathology score (ρ=0.57, p=0.07) and a better negative correlation between TTP/Peak enhancement and the same score with statistical significance (ρ=-0.76, p<0.01).

In the initial part of our study we found good correlations for UC between the Mayo endoscopic scores and the TIC volume parameters, better than the clinical score (Table III). Other parameters such as CRP and Thickness showed significant but less strong correlation, while TIC flow parameters (RT, TTP and mTTT) did not show a significant correlation. By the multivariate analysis, the TIC volume parameters were also identified as good independent predictors for the endoscopy score (Fig. 3). The best independent predictor was ln(AUC), significantly discriminating between the main classes of endoscopic score. Although not directly comparable due to their histopathological standard, the study of Girlich et al. [23] has shown a roughly similar correlation for Imax.

Similarly, for CD we found the best correlations (Table III) between SES-CD and the TIC volume parameters, while the CDAI clinical score (ρ =0.58) showed, together with CRP, Thickness and Layer score a significant although less strong correlation. Only two predictors were found significant for SES-CD assessment in the multivariate analysis – Imax and ln(AUC), with the latter discriminating the best between the main classes of the endoscopic score (Fig. 4). It is interesting to note that in CD, the clinical score had a lower performance while the Layer score and CRP had a better performance in assessing endoscopic activity, compared to UC. A similar relation concerning clinical vs endoscopic scores was described in the literature [34].

In the second part of our study we found that the best predictor for endoscopic improvement in both UC and CD was ln(AUC), with a Wilcoxon Z score of 3.76 and 2.61, respectively. There was also a good correlation between the difference of its values and the difference in endoscopic scores before and after the treatment (ρ=0.68 in UC and 0.73 in CD). Imax and AUC have similar performance, while the clinical scores (Z=2.86 for UC and Z=2.3 for CD) and Layer score show slightly lower statistical significance of the difference between investigations. Interestingly, in the case of the TIC flow parameters, as well as the wall thickness, there was a better performance in discriminating endoscopic improvement in the case of CD (with statistical significance for RT and mTT, as well as the thickness). CRP, on the other hand, performed worse in CD, being unable to show statistically significant differences before and after treatment.

One has to take into account some of the limits of our study. Other than the rather small population of followed-up patients (especially in CD, compensated statistically by the use of non-parametric tests), there might have been a recruitment bias, since all the patients have already been diagnosed with IBD. Nevertheless, the population ended up quite homogeneously distributed regarding demographics and disease activity (Table II). An important topic was the effectiveness of the comparison between CEUS and ileo-colonoscopy. Being blinded with regard to the endoscopic findings, the US examiner had to choose the most affected segment, that could have not corresponded with the most affected segment found by endoscopy. However, the intestinal loops located far from the ileocecal junction were not examined, which would have been inaccessible at endoscopy. On the other hand, endoscopy cannot assess the changes deep in the bowel wall structure or beyond, probably causing some discrepancies in scoring between the two methods. Another important limit of our study is the lack of reported possible interobserver variability, as all the US investigations were interpreted by the same person.

The post-processing of the TIC curves is an important factor in our study. We chose a 3rd party software (SonoLiver) for this task, as we wanted our results to be systematic and easily repeatable on other systems. An important emphasis was also laid on the choice of the reference organ (anterior abdominal muscles) and the selection of the region-of-interest (ROI). A revealing study by Ignee et al. [35] shows that the shape and size of ROI is not as important in quantification as the depth of ROI is. We could not control the depth of the lesions, but we always tried to measure on the anterior walls, as close to the reference ROI from the muscle. By choosing a free-hand ROI covering the entire wall is, in our experience, the best way for ensuring more data and less error from sampling from outside the wall (small movements are unavoidable during the clip). A final point to discuss concerning the TIC quantification is the difficulty encountered especially in patients with complete remission. Narrow walls and weak enhancement leads to low-quality TICs, so the results would be less predictable for those patients. Volume parameters (i.e. AUC) are less dependent on the shape of the curve, so they give better results than flow parameters, which vary widely in the same conditions [9].

CONCLUSION

We suggest that quantitative measurements of bowel enhancement by using contrast-enhanced US might be an alternative to the endoscopic scores in assessing both CD and UC activity and the response to treatment. The most promising CEUS parameter was AUC, measured in logarithmic form,

correlating better with the endoscopic activity than the clinical scores, CRP and other US parameters. Layer thickness still proved quite a valuable and easy method to identify non-responders to treatment. The CEUS could be a useful technique to monitor more frequently the activity in IBD patients during treatment, without the added discomfort and risks of ileo-colonoscopy.

Conflicts of interest. Nothing to declare.

Authors' contribution. M.S. and L.C.: study design, acquisition and interpretation of data, database creation, statistical analysis, and drafting of the manuscript; B.D.: data acquisition, database creation; C.H. and A.S.: study design, data acquisition; R.B.: concept of the study, supervision of the study and design, data acquisition.

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