Predictive value of time–intensity curves obtained with contrast-enhanced ultrasonography (CEUS) in the follow-up of 30 patients with Crohn's disease

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KEYWORDS

Crohn's disease;
Ultrasonography;
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CEUS;
SonoVue;
CDAI;
Disease activity.

Abstract *Objectives:* CEUS can provide accurate quantitative estimates of intestinal wall microvascularization in Crohn's disease. We hypothesized that inflammation of the intestinal wall is correlated not with the amount of wall vascularization (study of vascularization patterns, SVP) but with the degree of wall flow during a period of time (time–intensity study, TIS). Our objective was to discover whether CEUS SPV and/or CEUS-TIS reflect(s) vascular inflammation of the intestinal wall and display(s) correlation with clinical activity of the disease at the time of the examination (T0) or at the 3- and 6-month follow-up (T3, T6).

Materials and methods: 30 patients with Crohn's disease (12 men, 18 women, mean age: 41.96 years; treatment: 5-ASA ($n = 8$), steroids ($n = 13$), anti-TNF ($n = 7$), azathioprine ($n = 2$) were studied with CEUS SPV and CEUS-TIS and followed for at least 6 months. The sonographic examinations were performed with SonoVue (BR1, Bracco) and a dedicated scanner (TECHNOS MPX, Esaote) equipped with software for calculation of time–intensity curves. Four vascular patterns (1: vascularization of the entire wall; 2: vascularization of $>50\%$ of the wall; 3: flow exclusively within the submucosal layer; 4: no signal). The semiquantitative analysis consisted in measurement of the area under the curve (AUC) (cut-off between active and inactive disease, 15), mean intensity (IMA) (cut-off = 10). Each examination (180 s) was digitally recorded and analyzed.

Results: T0: cDAI <150 in 22 pts; cDAI >150 in 8; T3: 22 pts. with cDAI <150 , 8 with cDAI >150 . At T0 CEUS SPV and CEUS-TIS both displayed low specificity, diagnostic accuracy, and negative predictive values ($p = ns$). At T0, CEUS SPV produced 8 true positives (TP), 15 true negatives (TN), 8 false positives (FP), 0 false negative (FN) (sensitivity: 100%; specificity: 68.2%; diagnostic accuracy: 69.5%; Positive predictive value (PPV): 100%; negative predictive value (NPV: 53.3%), and CEUS-TIS produced 6 TP, 18 TN, 4 FP, 2 FN (sensitivity 75%; specificity: 81.8%; diagnostic accuracy: 75%; PPV: 60%; NPV: 90%). At T3, CEUS SPV produced 8 TP, 12 TN, 7 FP, 3 FN (sensitivity: 72.7%; specificity: 63.2%; diagnostic accuracy: 50%; PPV: 53.3%;

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NPV: 80%), and CEUS SIT produced the following results: 10 TP, 19 TN, 0 FP, 1 FN (sensitivity: 90,9%; specificity: 100%; diagnostic accuracy: 96,5%; PPV: 100%; NPV: 95%).

At T3 CEUS-SVP displayed low sensitivity and low diagnostic accuracy, whereas SIT was able to predict clinical activity during follow-up in all but one case (which showed reactivation after 6 months) ($p = 0.001$)

Conclusion: CEUS-TIS alone was found to reflect vascular inflammation of the intestinal wall in Crohn's disease and predicted clinical activity during follow-up.

Sommario *Obiettivi:* La CEUS è in grado di quantificare accuratamente la microvascolarizzazione della parete intestinale nel Crohn.

Ipotesi: L'infiammazione della parete intestinale non è correlata con la quantità di parete vascolarizzata (studi di pattern di vascolarizzazione - SPV), ma con il grado di flusso di parete durante un periodo di tempo (studi di intensità-tempo - SIT). Obiettivo è stato capire se gli studi SPV o quelli SIT con CEUS fossero espressione dell'infiammazione vascolare della parete intestinale e fossero correlabili con l'attività clinica di malattia (T0) o nel follow-up (tre e sei mesi: T3, T6).

Materiali e metodi: 30 Crohn (M: 12; F: 18; età media: 41,96; terapia: 8 Pts 5-ASA; 13 steroide; 7 ANTI-TNF; 2 azatioprina) sono stati seguiti per almeno sei mesi e studiati con CEUS SPV e con CEUS SIT. È stato utilizzato come mezzo di contrasto il SonoVue (BR1, Bracco) e un ecografo dedicato (TECHNOS MPX, Esaote) con software per curve di time intensity. Quattro pattern vascolari (1: tutta la parete vascolarizzata; 2: più del 50% di parete con vascolarizzazione; 3: flusso solo all'interno della sottomucosa; 4: nessun segnale). Un'analisi semiquantitativa è stata ottenuta misurando l'area sotto la curva - AUC (cut-off tra attività ed inattività = 15), l'intensità media - MI (cut off = 10). Tutti gli esami sono stati eseguiti per 150 secondi, registrati e analizzati in modo digitale. *Risultati:* T0: cDAI <150 in 22 pts; cDAI > 150 in 8; T3: 22 pts con cDAI <150, 8 con cDAI >150. A T0 sia la CEUS SPV che la SIT hanno ottenuto bassa specificità, accuratezza diagnostica e valore predittivo negativo ($p = ns$); la CEUS SPV ha dato a T0: 8 VP, 15 VN, 8 FP, 0 FN (sens: 100%; spec: 68,2%; acc diagn: 69,5%; VPP: 100%; VPN: 53,3%); la CEUS SIT ha conseguito a T0: 6 VP, 18 VN, 4 FP, 2 FN (sens: 75%; spec: 81,8%; acc diagn: 75%; VPP: 60%; VPN: 90%). La CEUS SPV ha raggiunto a T3: 8 VP, 12 VN, 7 FP, 3 FN (sens: 72,7%; spec: 63,2%; acc diagn: 50%; VPP: 53,3%; VPN: 80%). La CEUS SIT invece ha invece ottenuto i seguenti risultati: 10 VP, 19 VN, 0 FP, 1 FN (sens: 90,9%; spec: 100%; acc diagn: 96,5%; VPP: 100%; VPN: 95%).

A T3 la CEUS-SVP ha una bassa sensibilità e una bassa accuratezza diagnostica, mentre la SIT è in grado in tutti i casi eccetto uno (che riattiva dopo 6 mesi) di prevedere l'attività clinica durante il follow-up ($p = 0,001$).

Conclusioni: Solo la CEUS-SIT ha dimostrato di essere espressione dell'infiammazione vascolare della parete intestinale del Crohn e capace di predire, nel follow-up, il suo tipo di attività.

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Introduction

There is a substantial body of evidence demonstrating the value of B-mode ultrasonography [1–3] as a simple, noninvasive tool for diagnosing Crohn's disease [4], defining its extension, and detecting complications [5]. This disease is associated with intestinal neovascularization, as demonstrated by pathological findings [6] and by the presence of high serum levels of angiogenic factors [7]. The use of color [8] and power Doppler [9] techniques has revealed increased blood flow in the intestinal wall of patients with active disease, and the correlation between disease activity and intestinal wall vascularity has been confirmed by pulsed Doppler findings [8,10,11]. In previous studies [12–16], changes in disease activity were demonstrated by Doppler analysis of blood flow through the superior mesenteric artery. This approach has several limitations including strong operator-dependence, wide inter-patient variability, and inadequate visualization of the microvasculature of the gut wall [17].

Although various authors have reported their own experiences [18–22], there is no consensus on the use of

contrast-enhanced ultrasonography (CEUS) for assessing the activity of Crohn's disease, much less on its possible role as a short-term prognostic tool. Although both first- [18] and second-generation [19–22] ultrasound contrast agents can be used to visualize the microvasculature of the intestinal wall (study of wall vascularization patterns, SVP), a recent review [5] has reexamined the data from these studies and found that correlation between disease activity and studies of wall vascularization is associated with a 30% rate of false positives, which makes this approach unsuitable for use in clinical practice. However, in the literature, there are also reports of patients with clinically quiescent forms of Crohn's disease associated with wall vascularization, who develop recurrent disease after a short period of time.

Objectives

Based on our review of the available literature, we hypothesized that CEUS might be used to predict clinical recurrence of Crohn's disease based on semiquantitative measurements of blood flow within the intestinal wall. Flow

is measured with specialized software capable of evaluating the intensity over time of microflow within the intestinal wall (time–intensity study, TIS). This prospective study was based on the etiopathogenetic hypothesis that inflammation of the intestinal wall is not correlated with the degree of vascularization (which can be assessed with wall vascularization studies, i.e., SVP) but with the amount of blood flowing through these vessels (time–intensity studies, TIS). The aim of this prospective study was to evaluate the activity of the vascularization within the wall using CEUS in order to correlated wall vascularization (CEUS-SVP) or the intensity of flow over time (CEUS-TIS) with clinical activity at the time of the examination or during short-term follow-up (3–6 months after CEUS).

Materials and methods

We prospectively enrolled 30 consecutive patients with histologically confirmed Crohn's disease (12 men, 18 women, mean age 41.9 years, range 24–71 years). Their clinical characteristics are summarized in Table 1.

Diagnosis of the disease had been based on biopsy findings, and all patients were examined with colonoscopy within 1 month of CEUS.

A few days before the CEUS examination, blood was drawn for complete blood counts, erythrocyte sedimentation rates, and C-reactive protein levels, and patients completed a questionnaire on the clinical features of their disease (abdominal pain, fever, number of bowel movements per day, presence of blood in the feces). Based on the patient history, the results of the physical examination, and available laboratory data, the Crohn's Disease Activity Index (CDAI) [23,24] and the simplified Disease Activity Index (DAI) [25] were calculated for each patient.

Ultrasonographic examination

Before CEUS, patients underwent conventional abdominal sonography. The examination was performed with a TECHNOS MPX scanner (Esaote, Italy) and a convex 3.5-MHz transducer. Later, the intestinal loops were reexamined with a linear 7.5-MHz transducer. The examination focused on the diseased portion of the intestine (ileum or colon) or the segment considered to be affected by active disease at the time of the examination, based on the location of pain and the sonographic features. In patients whose disease was clinically silent, we examined the diseased portion of the intestine, or when multiple segments were involved, the segment displaying maximal wall thickness. For each segment examined, we recorded wall thickness, presence/absence of all 3 wall layers, and the presence of fluid collections and/or fistulas. Power Doppler was used to evaluate the presence of blood flow within the wall or in the surrounding mesentery, and findings were expressed using the semiquantitative scale described by Esteban [8] (0: absence of flow = inactive disease; 1: scarce signal = limited activity; 2: high-intensity signal = active disease). Pulsed Doppler was used to measure the resistance index (RI). The cut-off used to distinguish active from inactive disease was 0.65 [8].

Table 1 Clinical characteristics of the patients.

	Total N = 30	Men N = 12	Women N = 18
Mean age (years)	41.96	43	41.3
Range	24–71	28–71	24–69
Site of disease			
Ileum	6	4	2
Ileum–colon	19	6	13
Colon	5	1	4
Disease type			
Stenotic	8	6	2
Fistulizing	11	5	6
Inflammatory	11	6	5
Crohn's disease activity index			
CDAI < 150	22	10	12
CDAI > 150	8	1	7
Simplified disease activity index (DAI)			
Remission	20	9	11
Mild activity	2	1	1
Moderate activity	7	1	6
Severe activity	1	0	1
Previous surgery			
NO	22	5	17
YES	8	6	2
Treatment			
5-ASA	8	4	4
Cortisone	13	2	11
Anti-TNF	7	4	3
Azathioprine	2	1	1

Contrast-enhanced ultrasonography

CEUS was performed with a second-generation contrast agent, Sonovue (Bracco, Italy) and a dedicated scanner (Technos MPX, Esaote, Italy) with second tissue harmonics in gray scale. With this technique, signals originating from the microbubbles are separated from those produced by tissues. The US signal is separated and elaborated according to signal-frequency cut-offs. This means that the scanner emits a small wide-band signal and then selectively filters the returning signals to isolate the second harmonic. This produces an image consisting exclusively of signal produced by the microbubble contrast agent. The use of low acoustic power eliminates the risk of bubble rupture, and all vascular phases can be analyzed in real time. This technique has been referred to as *perfusional angiosonography* to highlight its dynamic nature.

Each patient received a 4.8-mL bolus of SonoVue, which is a higher dose than that normally used for the study of focal hepatic lesions [26]. The increased dose was used because the examination required prolonged perfusion and because the transducer we used employs relatively high mechanical indexes, which increase the rate of bubble rupture.

As described in the literature [27], CEUS patterns for the diseased intestinal segment were divided into four types: Pattern 1 (single layer), which is characterized by intense, full-thickness enhancement of the intestinal wall; Pattern 2 (double layer), characterized by enhancement confined to the mucosal and submucosal layers and sparing of the external muscle layer; Pattern 3 (triple layer), in which the mucosal and muscle layers remain unenhanced and enhancement is seen in the submucosal layer; and Pattern 4, which is characterized by nonenhancement and indicative of wall scarring (Fig. 1). Patterns 1 and 2 are indicative of clinically active disease; patterns 3 and 4, inactive disease.

Time–intensity curves

The region of interest was scanned for 180 s after injection of the contrast agent. The scanner screen displays the exact number of seconds that have elapsed since the injection. The scanner automatically records the examination (video recording and digital recording).

While the examination was being recorded, the scanner acquired data for the elaboration of time–intensity curves [28–31], which were then analyzed off-line with IMAGE-LAB software (Esaote, Italy). Regions of interest (ROI) of the

same caliber were then positioned. The ROIs evaluated the entire intestinal wall. The curves obtained represent the sum of the signals for each pixel for the duration of acquisition of the sample volume based on the pre-established duration of acquisition.

The entire examination was reviewed with point-by-point analysis of the time–intensity curve and analysis of corresponding CEUS images. This furnished simultaneous graphic and morphologic assessments of the transit of the contrast agent. The software automatically calculated several parameters, including the total area under the curve (AUC), the AUC during the diastolic phase (AUC_{diast}), mean intensity of the AUC (IMA); the slope of the segment between the origin and the systolic peak (syst slope) and that of the segment between the systolic peak and the diastolic peak (diast slope).

The AUC represents the amount of contrast agent that passes through the area within the predefined interval of time, and it is an expression of blood flow during the quantization study. The AUC_{diast} reflects blood flow through the intestinal wall in the portal and late phases. The IMA represents the mean amount of flow recorded during the examination. The 2 slopes indicate filling velocity in the arterial phase (slope syst) and emptying during the portal and late phases (slope diast).

Fig. 1 1A–D: CEUS examination of a patient with active Crohn's disease: A: The baseline examination shows a richly vascularized intestinal wall; B–D: CEUS-SVP revealed intestinal wall vascularization that was detectable in all phases; this vascularization persists in the late phase (D) (CEUS pattern 1). 2 A–D: Patient with active Crohn's disease whose power Doppler study revealed less intense vascularization (2A): (CEUS pattern 2). 3 A–D: Patient with inactive Crohn's disease: power Doppler (3-A) shows no vascularization; the CEUS (3-B–D) examination produced Pattern 3 findings. 4 A–D: Patient with inactive Crohn's disease: power Doppler (4-A) shows no vascularization; at CEUS (4-B–D), almost no vascularization was seen. 1–4 E: Corresponding time–intensity curves.

Follow-up

Follow-up visits were scheduled 3 and 6 months after CEUS (T3 and T6). Each visit included a physical examination, conventional ultrasonography, and assessment of the same laboratory parameters assessed before CEUS (time zero, T0). Findings at T3 (and when doubts arose also those at T6) were analyzed in light of the results of the patient's CEUS examination at T0.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy were calculated for CEUS patterns and time–intensity curves in predicting disease activity indexes at the time of the examination (T0) and at the 3-month follow-up visit (T3). Data were analyzed with Student's *t* test. In all analyses, a *P* value < 0.05 was considered statistically significant. Analyses were carried out with the aid of SPSS 13 software.

Results

At the time of the examination, 22 patients had CDAls < 150 (corresponding DAIs: 0–3 in 20 patients, 4–6 in 2), and 8

had CDAls > 150 (corresponding DAIs: 7–10 in 7 patients, 11–12 in 1). Nineteen of the patients (63.3%) had ileocolonic disease, 6/30 (20%) had ileal disease, and in 5/30 (16.7%) the disease was confined to the colon.

Table 2 shows the sonographic findings compared with the two main indexes of clinical activity at the time of the sonographic examination. Mean intestinal wall thickness measured during the conventional sonographic examination was higher in the group with active disease (8.1 vs. 7.1 in those with inactive disease). The typical three-layered wall structure was seen in 20 patients, 14 of whom (70%) had inactive disease. Of the ten patients whose intestinal walls appeared hypoechoic, only 2 (20%) presented clinical signs of active disease. None of the patients had free fluid in the peritoneal cavity; lymphadenopathy was observed in three patients with active disease and internal fistulas, as previously described in the literature [32]. Power Doppler studies revealed vascular signals in 23 patients, but rich wall vascularization could be found in only four.

Resistance indices > 0.65 were found in 13 patients with CDAls < 150, <0.6 in the clinically active patients and in 6 of the clinically inactive patients who had recurrence during follow-up. This finding presented a statistically significant difference (*p* < 0.05) in distinguishing vascularization of an active loop from an inactive one; at CEUS-SVP vascular patterns 1 and 2 (which indicate more extensive vascularization) were observed in all 8 patients with clinically active

Table 2 Sonographic findings and clinical indices of disease activity in the 30 patients with Crohn's disease.

			Disease activity (CDAI)			Disease activity (DAI)			
			TOT	Remission	Active	Remission	Mild	Moderate	Severe
			(CDAI < 150)		(CDAI > 150)	(0–3)	(46)	(7–10)	(11–12)
B-MODE	Intestinal involvement	Ileal	6	4	2	4	0	2	0
		Colonic	5	2	3	2	0	3	0
		Ileocolonic	19	16	3	14	2	2	1
	Triple-layered wall structure	Present	20	14	6	12	2	5	1
		Absent	10	8	2	8	0	2	0
	Mean wall thickness (mm)		7.4	7.1	8.1	7.2	6.5	8.2	7
Power	Vascularization	Absent	7	7	0	6	1	0	0
		Moderate signal intensity	19	13	6	12	1	5	1
		High signal intensity	4	2	2	2	0	2	0
Pulsed Doppler		MEAN R.I.	0.8	0.72	0.52	0.72	0.72	0.53	0.56
CEUS	Wall vascularization studies (WVS)	Pattern 1	3	1	2	0	1	2	0
		Pattern 2	12	6	6	6	0	5	1
		Pattern 3	13	13	0	12	1	0	0
		Pattern 4	2	2	0	2	0	0	0
	Time–intensity study (TIS)	AUC (mean)	12.4	10.9	16.5	10.98	10.32	16.84	12.41
		AUC DIAST (mean) ^a	12.3	11.4	15	11.7	13.4	14.9	14.39
		IMA (mean) ^a	8.5	7.6	11	7.7	6.67	11.2	9.65
		SLOPE SYST (mean) ^a	11	10.8	11.6	11.14	6.7	11.6	0 ^b
	SLOPE DIAST (mean) ^a	12.5	11.8	14.5	12.2	6.5	14.5	0 ^b	

CDAl, Crohn's Disease Activity Index [23,24]; DAI, simplified disease activity index [25].

^a In 10 patients, the systolic peak could not be measured.

^b Null value because there was no systolic peak.

Fig. 2 CEUS AUC values < 15 all were associated with clinical remission during the short-term follow-up, whereas higher AUCs were predictive of clinically detectable active disease during follow-up. Only one patient with an AUC < 15 experienced reactivation during follow-up (large yellow circle).

disease at the time of the examination and in 7 of those with inactive disease. Only 2 of 7 patients presented signs of active disease during follow-up. Patterns 3 and 4 (which indicate less extensive vascularization) were observed in 15 patients with active disease at the time of the examination, and 3 of these (20%) experienced flare-ups during follow-up. This finding demonstrates that the patterns observed at CEUS-SVP cannot be used to distinguish patients whose Crohn's disease is currently active or those who will experience reactivation during follow-up, although a progressive increase in less vascularized patterns was observed among the inactive patients ($p = ns$).

CEUS-TIS (performed at T0 time) revealed mean AUCs of 10.9 (range: 2.9–19) in patients with inactive disease at the time of the examination and 16.5 (range: 10.4–23.5) in those with active disease. The mean IMAs for these two subgroups were respectively 7.2 (range: 1.6–10.81) and 11 (range: 7–15.6).

The AUC values obtained from CEUS-TIS at T0 were analyzed, and threshold values were identified that could be used to predict active vs. inactive disease during follow-up (T3 or T6). The AUC cut-off for inactive disease was 15. (Only one patient with an AUC slightly lower than 15 presented signs of disease activity during the follow-up) (Fig. 2). As for the IMA, the threshold was 10. (The same patient presented a threshold value slightly less than 10 with activity during the follow-up) (Fig. 3). The other indicators were not considered because in one third of the patients (10/30), systolic peaks could not be identified, and consequently the AUC diast, slope syst, and slope diast could not be calculated.

CEUS-TISs were then reviewed in light of the pre-established cut-offs. When patients were stratified by CDAI (Table 3) and DAI (Table 4) recorded at T0 (the time of CEUS), we found that 90% (18/20) of those with CDAs indicative of inactive disease (and 85% [17 of 20] of those with DAIs reflecting inactive disease) had AUCs < 15 and IMA < 10 at CEUS; AUCs > 15 (and corresponding IMAs > 10) were found in the majority of those with active disease (75% [6/8] based on the CDAI; 66.6% [6/9] based on the DAI) but also in a non-negligible proportion of those with inactive disease (22.2% [4/18] based on the CDAI; 17.6% [3/17] based on the DAI).

At the 3-month follow-up visit, 22 patients were in clinical remission and 11 had clinical evidence of active disease. Five (45.45%) of these 11 patients had had CDAs and DAIs at the time of CEUS (T0) that were indicative of inactive disease. In all cases, signals were observed on power Doppler, but the RI was > 0.65 . In three, the CEUS pattern was also indicative of inactive disease (Pattern 3), but the other two had patterns associated with active disease (Pattern 1 in one case, Pattern 2 in the other). Analysis of the CEUS-TISs showed that 4 of the 5 had AUCs > 15 and IMAs > 10 . The fifth patient had AUC and IMA values that were slightly lower than the predefined cut-offs. The other six patients with active disease at T3 had also presented active disease at T0. Only 2 of these presented Doppler signals indicative of vascularization, and only 3 had CEUS patterns indicative of active disease (Pattern 1). Analysis of the CEUS-TISs revealed AUCs > 15 and IMAs > 10 in all six.

Fig. 3 CEUS IMA values < 10 all were associated with clinical remission during the short-term follow-up, whereas higher IMAs were predictive of clinically detectable active disease during follow-up. Only one patient with an IMA < 10 experienced reactivation during follow-up (large yellow circle); she is the same one who also had an AUC < 15 (see Fig. 2).

Table 3 Correlation between CEUS findings at T0 with disease activity status estimated by the CDAI at T0 and T3.

CEUS findings at T0	Disease activity status based on CDAI			
	T0		T3	
	Remission N = 22	Active N = 8	Remission N = 19	Active N = 11
Pattern				
1	1	2	0	3
2	6	6	7	5
3	13	0	10	3
4	2	0	2	0
Time–intensity curve				
AUC < 15	18	2	19	1
IMA < 10				
AUC > 15	4	6	0	10
IMA > 10				

Table 5 shows the prognostic value of CEUS-SVP and CEUS-TIS in predicting the activity status of Crohn's disease reflected by CDAI or DAI calculated at the time of CEUS and at the 3-month follow-up.

CEUS (SVP) displayed high sensitivity (100%), but low specificity (62%) in predicting disease activity at the time of the examination (T0). For predicting disease status during the follow-up (T3), its diagnostic accuracy was low. In contrast, CEUS-TIS was neither highly sensitive nor highly specific in predicting disease status at the time of the examination, but its overall accuracy in predicting disease status at the 3-month follow-up (T3) was 96.5% (T3).

Discussion

There is currently no gold-standard reference method for assessing the activity status of Crohn's disease. The best known method, the CDAI, has been widely discussed, and it is still the object of criticisms although it has been adopted as the reference method in many important clinical trials [33–38]. Other scoring systems like the DAI have also been used, but none of these approaches offers optimal staging of this disease, much less reliable predictions of short-term outcomes.

There are no clinical, biohumoral, or instrumental systems that can be used during follow-up to predict the activity of the disease in a clinically inactive patient with wall vascularization.

The use of ultrasonography, a noninvasive method, has revealed correlation between the inflammatory activity of Crohn's disease and the degree of vascularization. The latter variable has been measured in various ways, including calculation of the RI in the superior mesenteric artery [12–16], analysis of the RIs of arteries located within the diseased intestinal loop [8–11,18], and with the use of contrast-enhanced power Doppler performed with first and second-generation ultrasound contrast agents. None of these approaches, however, provides information on the microvasculature.

The recent introduction of second-generation ultrasound contrast agents has made it possible to study the microvasculature of the intestinal wall. Thus far, three studies have been conducted to evaluate the correlation between ultrasound contrast agent and disease activity. They showed that intense wall enhancement is found in 36.5% (19/52) [19] to 38.6% (8/21) [20] of patients with active disease but also in 26.6% (4/15) [22] of those in remission. In our experience, 46.6% (7/15) of patients with clinical remission presented substantial wall vascularization. Because of this confounding element, Maconi et al. [5] maintain that the correlation between vascularization of the diseased intestinal loop revealed by CEUS and the activity of Crohn's disease is of limited value. An additional confirmation has emerged from a semiquantitative study of wall vascularization [20], which found no correlation between the CDAI and the quantitative estimate of wall vascularization. This same picture emerges from our experience: 30% (3/10) of the patients whose TISs were indicative of active disease ($AUC \geq 15$; $IMA \geq 10$) were in clinical remission. Therefore, CEUS also provides an inadequate representation of disease activity, particularly in quiescent forms of Crohn's disease (as reflected by its low specificity). Similar findings were presented by Heyne and coworkers [10]. They found that the presence of flow detected with color Doppler was correlated with disease activity in patients with ulcerative colitis, but no such correlation was observed in those with Crohn's disease. Correlation between local vascularization of the diseased intestinal loop and disease activity assessed by means of the CDAI remains contradictory for all imaging, endoscopic,

Table 4 Patterns observed at CEUS-SVP and CEUS-TIS parameters as functions of disease activity expressed by the DAI.

DAI					
T0			T3		
CEUS pattern	Remission	Activity	Pattern CEUS	Active	Inactive
1	0	3	1	3	0
2	6	6	2	5	7
3	12	1	3	3	10
4	2	0	4	0	2
Area under the curve	Remission	Activity	Area under the curve	Remission	Activity
<15 (IMA < 10)	17	3	<15 (IMA < 10)	19	1
>15 (IMA > 10)	3	7	>15 (IMA > 10)	0	10

Table 5 Prognostic value of CEUS-SVP and CEUS-TIS in predicting the activity status of Crohn's disease reflected by CDAI or DAI calculated at the time of CEUS and at the 3-month follow-up.

Reference	Activity status		CEUS-SVP results				CEUS-SVP accuracy				
	Active	Inactive	True+	False+	False–	True–	SENS	SPEC	ODA	PPV	NPV
CDAI AT T0	8	22	8	7	0	15	100	68.2	69.5	53.3	100
DAI AT T0	10	20	9	6	1	14	90	70	69.5	60	93.3
CDAI AT T3	11	19	8	7	3	12	72.7	63.2	50	53.2	80
DAI AT T3	11	19	10	0	1	19	90.9	100	95.5	100	95
	Activity status		CEUS-TIS results				CEUS-TIS accuracy				
	Active	Inactive	True+	False+	False–	True–	SENS	SPEC	ODA	PPV	NPV
CDAI AT T0	8	22	6	4	2	18	75	81.8	75	60	90
DAI AT T0	10	20	7	3	3	17	70	85	75	70	85
CDAI AT T3	11	19	10	0	1	19	90.9	100	96.55	90.9	100
DAI AT T3	11	19	10	0	1	19	90.9	100	96.55	90.9	100

Abbreviations: Sensitivity (SENS), specificity (SPEC), overall diagnostic accuracy (ODA), positive predictive value (PPV), negative predictive value (NPV), CEUS with study of wall vascularization patterns (CEUS-SVP), CEUS with Time Intensity studies (CEUS-TIS).

and radiological techniques (CT [39,40]; MRI [41,42]). However, in daily practice, it is frequently observed that the presence of intestinal wall vascularization in patients whose Crohn's disease is quiescent may be an indicator of imminent reactivation. Our pathogenetic hypothesis, to obtain semiquantitative (but in any case "objective") measurements of intraparietal blood flow in patients with Crohn's disease confirmed that vascular activity cannot be correlated with clinical activity, but its measurement is useful in clinical practice. In fact, our experience revealed that in the subgroup of patients with clinically inactive disease by wall vascularization typical of inflammation, clinically relevant recurrence is present at the 3-month follow-up visit. This finding is statistically significant ($p < 0.001$) and associated with diagnostic accuracy of 96.5% (with sensitivity greater than 90% and specificity of 100%). A similar finding has been reported by De Pascale and coworkers [22]. They described a subset of 4/15 patients with clinically inactive disease at the time of CEUS, in whom vascularization was present and clinical recurrence was observed approximately 3 months later. These authors also reported a false positive. In our experience there was only one false negative, that is a patient with clinically silent disease who developed recurrence during follow-up despite the fact that her AUC and IMA were slightly below the cut-off.

The number of considered patients is obviously a limitation of this series, and for this reason further confirmatory studies are necessary. Another limitation is related to the segment of the intestine studied. However, it is important to consider that in such patients one already knows the site or sites of involvement, and when there is multiple-site involvement, different sonographic assessments can be carried out.

In conclusion, our findings confirm the use of CEUS in the evaluation of the short-term prognosis of disease (3–6 months) and they seem to be capable of predicting possible recurrence of the disease within the near future. This would allow wider use of this method in the follow-up of patients with Crohn's disease.

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Conflict of interest statement

The authors have no conflict of interest.

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