Radiology

Time-Intensity Curves Obtained after Microbubble Injection Can Be Used to Differentiate Responders from Nonresponders among Patients with Clinically Active Crohn Disease after 6 Weeks of Pharmacologic Treatment[1]

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Purpose: To assess whether contrast material–enhanced ultrasonography (US) can be used to differentiate responders from nonresponders among patients with clinically active Crohn disease after 6 weeks of pharmacologic treatment.

Materials and Methods: This prospective study was approved by our ethics committee, and written informed consent was obtained from all patients. Fifty consecutive patients (26 men and 24 women; mean age, 34.76 years ± 9) with a proved diagnosis of active Crohn disease who were scheduled to begin therapy with biologics (infliximab or adalimumab) were included, with enrollment from June 1, 2013, to June 1, 2015. In each patient, the terminal ileal loop was imaged with contrast-enhanced US before the beginning and at the end of week 6 of pharmacologic treatment. Time-intensity curves obtained in responders (those with a decrease in the Crohn disease endoscopic index of severity score of 25–44 before treatment to 10–15 after treatment, an inflammatory score <7, and/or a decrease ≥70 in the Crohn disease activity index score compared with baseline) and nonresponders were compared with Mann-Whitney test.

Results: Responders (n = 31) and nonresponders (n = 19) differed (P < .05) in the percent change of peak enhancement (−40.78 ± 62.85 vs 53.21 ± 72.5; P = .0001), wash-in (−34.8 ± 67.72 vs 89.44 ± 145.32; P = .001) and washout (−5.64 ± 130.71 vs 166.83 ± 204.44; P = .002) rate, wash-in perfusion index (−42.29 ± 59.21 vs 50.96 ± 71.13; P = .001), area under the time-intensity curve (AUC; −46.17 ± 48.42 vs 41.78 ± 87.64; P = .001), AUC during wash-in (−43.93 ± 54.29 vs 39.79 ± 70.85; P = .001), and AUC during washout (−49.36 ± 47.42 vs 42.65 ± 97.09; P = .001). Responders and nonresponders did not differ in the percent change of rise time (5.09 ± 49.13 vs 6.24 ± 48.06; P = .93) and time to peak enhancement (8.82 ± 54.5 vs 10.21 ± 43.25; P = .3).

Conclusion: Analysis of time-intensity curves obtained after injection of microbubble contrast material 6 weeks after beginning pharmacologic treatment can be used to differentiate responders from nonresponders among patients with clinically active Crohn disease.

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In patients with Crohn disease, especially in clinical trials, the therapeutic outcome of pharmacologic treatment is assessed with the Crohn disease activity index (CDAI) (1–3). Unfortunately, the CDAI has low specificity because it is mainly based on subjective symptoms, whereas the adjustment of pharmacologic treatment should be based on objective measures of inflammatory activity to avoid inappropriate prolonged treatment (3). Corticosteroids and/or biologic drugs are effective in treating patients with clinically active Crohn disease, even though biologic drugs are expensive, present potential toxicities, and often fail to reduce Crohn disease activity (4). Hence, a reliable and early assessment of the pharmacologic effect is necessary to identify patients who respond to the treatment (5).

Endoscopy (ileocolonoscopy) represents the reference standard for the assessment of Crohn disease inflammatory activity (5). However, endoscopy does not show the transmural extent of Crohn disease, and intubation of the distal ileum can be completed in only 80% of patients with Crohn disease (1,2). Computed tomography (CT) and magnetic resonance (MR) imaging can depict the transmural and extraintestinal extent of Crohn disease, even though they require large amounts of enteric contrast material (6–12). Moreover, CT exposes the patient to high radiation doses, especially if it is repeated during a strict follow-up schedule, as in patients who undergo surveillance during pharmacologic treatment.

Previous studies showed the capabilities of contrast material–enhanced ultrasonography (US) for classifying Crohn disease severity and differentiating responders from nonresponders with time-intensity curve analysis before and after beginning pharmacologic treatment (13–18). In particular, a recent article proposed an early evaluation of time-intensity curves after microbubble injection (at 1 month and at 3 and 12 months after initiation of the treatment) in patients with Crohn disease undergoing pharmacologic treatment, while usually the clinical assessment of therapeutic outcome is performed at 12 weeks (18). In this study, only the absolute values of the different kinetic parameters were compared, whereas the percent change of each kinetic parameter before versus after beginning pharmacologic treatment, which could represent a better method of differentiating responders from nonresponders and reduce the wide interpatient variability, was not assessed (18). Moreover, analysis of a larger patient population with identification of potential thresholds for each kinetic parameter to differentiate responders from nonresponders is needed to avoid prolonged expensive and ineffective treatments in patients who have no chance to respond to treatment. The aim of this study was to assess whether contrast-enhanced US can be used to differentiate responders from nonresponders among patients with clinically active Crohn disease after 6 weeks of pharmacologic treatment.

Advances in Knowledge

- Patients with Crohn disease who respond to therapy can be differentiated from nonresponders on the basis of the percent change of peak enhancement (−40.78 ± 62.85 vs 53.21 ± 72.5; $P < .05$) as measured before and 6 weeks after beginning pharmacologic therapy.
- Patients with Crohn disease who respond to therapy can be differentiated from nonresponders on the basis of the percent change of the area under the time-intensity curve (AUC) (−46.17 ± 48.42 vs 41.78 ± 87.64; $P < .05$), AUC during wash-in (−43.93 ± 54.29 vs 39.79 ± 70.85; $P < .05$), and AUC during washout (−49.36 ± 47.42 vs 42.65 ± 97.09; $P < .05$) as measured before and 6 weeks after beginning pharmacologic therapy.

Implications for Patient Care

- Contrast-enhanced US may become useful for monitoring the efficacy of specific pharmacologic therapies in patients with Crohn disease.
- Adjusting pharmacologic treatment on the basis of contrast-enhanced US findings may help avoid inappropriate prolonged treatment with expensive biologic drugs.

Materials and Methods

Patients

This prospective observational study was approved by the ethics committee of our institute, and written informed consent was obtained from all patients. During a 24-month period (from June 1, 2013, to June 1, 2015), we recruited all patients with a biopsy-proved diagnosis of Crohn disease, a history of Crohn disease with disease activity from a maximal time period of 3 months, and involvement of the terminal ileum at endoscopy with serial deep mucosal biopsy who were scheduled to undergo biologic drug therapy (infliximab or adalimumab) either with or without corticosteroids to reduce Crohn disease activity in the acute phase as planned by the referring gastroenterologist. The following inclusion criteria were used: *(a)* age greater than 18 years and *(b)* moderately or severely active Crohn

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Abbreviations:
AUC = area under the whole time-intensity curve
CDAI = Crohn disease activity index
ROI = region of interest

Author contributions:
Guarantor of integrity of entire study, E.Q.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, R.A., A.G.G.; clinical studies, E.Q., M.S., A.G.G.; statistical analysis, E.Q.; and manuscript editing, E.Q., M.A.C.

Conflicts of interest are listed at the end of this article.

Figure 1

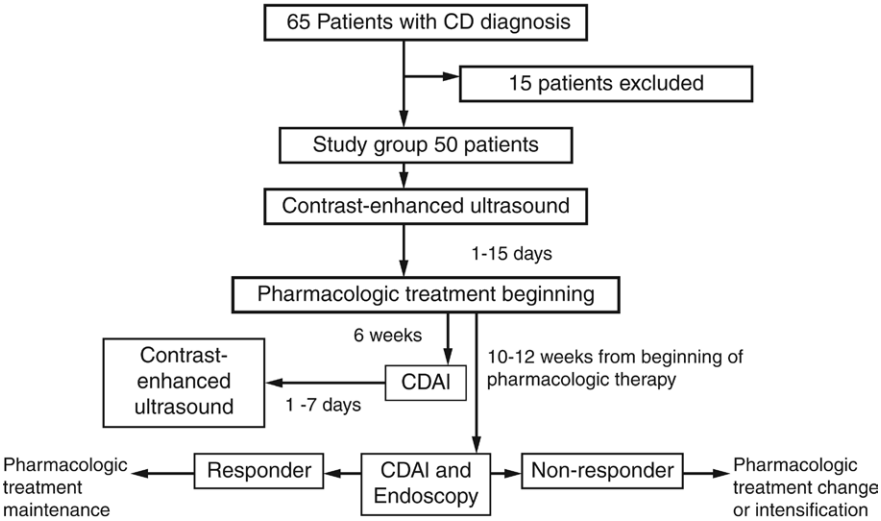

Figure 1: Flow diagram shows the study group. *CD* = Crohn disease.

Table 1

Characteristics of Responders versus Nonresponders

Characteristic	Responders	Nonresponders	*P* Value
Age (y)*	32.74 (22–65)	30.94 (19–42)	.02
Men	35.24 (22–65)	…	...
Women	34.74 (19–52)	…	...
Sex			…
Male	15	11	.57
Female	16	8	.57
Body mass index*	22.1 (20–24)	22.3 (20–26)	.6
Disease type			
Nonstricturing and nonpenetrating	5	5	.99
Structuring	22	13	.51
Penetrating	4	1	…
Disease duration (y)*	6 (1–10)	6 (1–10)	.99
Active disease duration (mo)*	2 (1–3)	2 (1–3)	.99
Disease location			
Ileum	31	15	.001
Ileo-colon	0	4	.008
Medication			
Biologics and corticosteroids	15	15	.99
Infliximab	8	9	…
Adalimumab	7	6	…
Adalimumab only	3	2	.7
Infliximab only	13	2	.057

Note.—Unless otherwise indicated, data are numbers of patients.

* Data are the mean, and data in parentheses are the range.

disease involving the terminal ileal loop as seen at endoscopy and/or with a CDAI score greater than 220 (19).

Sixty-five patients were initially included in this study. Fifteen of these patients were finally excluded because of unfeasible intubation of the distal ileum at ileum colonoscopy and, for this reason, were not classified as being a responder or a nonresponder (n = 2); ileal disease not appreciable at US (n = 2), corresponding to wall thickness of the terminal ileal loop of less than 3 mm; involvement of ileal tracts other than the terminal tract (n = 5); and insufficient clinical follow-up (< 12 weeks calculated after beginning pharmacologic treatment, with the day on which the new treatment was begun considered day 0; n = 6). Thus, 50 patients (26 men and 24 women; mean age, 34.76 years ± 9) with a history of active Crohn disease of 1–3 months (mean, 2 months) and ileum (n = 46) or ileum and colon (n = 4) involvement were included in the study (Fig 1). These patients underwent specific anti-inflammatory treatment on the basis of whether they underwent biologic drug (n = 20 [infliximab, n = 15; adalimumab, n = 5]) or biologic drug and corticosteroid (n = 30 [infliximab, n = 17; adalimumab, n = 13]) therapy (Table 1). Among patients who underwent biologic drug and corticosteroid therapy from the beginning of disease activity (n = 30), 19 were not taking steroids at the 6th week, while 11 were still taking low-dose corticosteroids (10–15 mg/day of prednisone).

US Examination

From 1 to 15 days before the beginning and within 7 days after the end of week 6 of pharmacologic treatment (42–49 days), each patient underwent US of the terminal ileal loop. After a fast of at least 6 hours, the terminal ileal loop was imaged by a diagnostic radiologist with 10 years of experience in abdominal US before and after intravenous administration of a single bolus of 4.8 mL of sulfur hexafluoride–filled microbubbles (SonoVue; Bracco, Milan, Italy) into a peripheral arm vein (duration of 1–2 seconds) followed by a 10-mL 0.9% saline flush.

US was performed with a broadband 256-element linear-array transducer (L12–5 and 5–12 MHz, 50 × 10 mm; Philips Healthcare, Bothell, Wash) and an iU22 xMATRIX US System (Philips).

This linear transducer was selected because it produces high-resolution rectangular images that are suitable for the following quantitation procedure. Before administering US contrast material, the termimal ileal loop wall thickness was measured (in millimeters) at the level of the anterior wall, the depth of the ileal loop was measured from the leading serosa edge of the anterior bowel wall up to the skin surface (in centimeters), and the absolute extension of the ileal tract involved was measured from the beginning to the end of the involved ileal tract by the thickening of the anterior bowel wall (in centimeters).

Contrast-enhanced US was performed by setting the following technical parameters at the same value before and after beginning pharmacologic treatment: power modulated pulse inversion technology; mechanical index, 0.08; dynamic range, 65 dB; 10–13 frames per second; echo-signal gain below noise visibility; signal persistence turned on; and one focus below the level of the small bowel segment. One uncompressed digital imaging and communications in medicine multiframe cine clip (15 frames per second and 2 minutes in duration) was acquired while the patient was breathing and transferred to the picture archiving and communication system in the radiology department.

Quantitative Analysis of US Images

One reference radiologist (E.Q., with 15 years of experience in abdominal contrast-enhanced US) performed image quantitative analysis of echo power by using a proprietary software package (VueBox, Version 4.3; Bracco, Geneva, Switzerland). Three sessions of analysis, each lasting 3 days, were necessary to complete the analysis. To assess interreader variability, two other blinded independent readers (R.A. and A.G.G., with 5 and 10 years of experience in abdominal imaging and contrast-enhanced US, respectively), performed quantitative analysis in a subgroup of 20 patients who were selected by simple randomization.

Each digital cine clip was transferred to a personal computer (Pentium 4; Intel, Santa Clara, Calif) that was connected to the picture archiving and communication system and was used for quantitative analysis. If peristalsis-related movements were evident in the imaged volume, the frames that appeared off-site to the reader were virtually excluded from quantitative analysis. Out-of-plane images and images that preceded the arrival of contrast material in the bowel wall were excluded from processing both by the reference radiologist and independent readers.

VueBox (Bracco) is used to linearize compressed digital imaging and communications in medicine Joint Photographic Experts Group-formatted images before performing curve fitting and analysis through application of an antilog function within the linear range of the microbubble concentration versus video-intensity relation. In each image, a manually defined polygonal (range, 4100–40 110 pixels; mean, 21 430 pixels) region of interest (ROI) that encompassed the thickened ileal tract over the anterior wall and avoided the lumen, or that covered the entire bowel wall if the lumen was not visible and avoided the mesentery and artifacts along the entire extension of the terminal ileum, was drawn and included in the screen field of view (Fig 2). A second ROI, which served as a potential internal reference and encompassed the adjacent mesentery and excluded the ileal wall, was drawn. The US video intensity was measured in linear arbitrary units, with the video intensity of pixels contained in each ROI expressed as mean plus or minus standard deviation. After the arrival of contrast material was automatically detected, time-intensity curves were fitted with a nonlinear least-squares regression method to a proprietary lognormal model (20,21). The quality of fit between the echo power signal and the theoretical curve was quantified with the least-square method.

The following kinetic parameters (Fig 3) were automatically calculated for each patient: peak enhancement, rise time (time to peak enhancement minus the time at which the maximum slope tangent intersects the x-axis), time to peak enhancement, wash-in rate (maximum slope, tangent at the ascending part of the curve), washout rate (minimum slope, tangent at the descending part of the curve), AUC, wash-in perfusion index (AUC during wash-in divided by rise time), AUC during wash-in, and AUC during washout. Data outliers were included in the analysis. In each patient, the percent change (post – pre $\times$ 100/pre, with "pre" and "post" referring to the linear value of each parameter measured in the bowel wall and adjacent mesentery before and 6 weeks after beginning pharmacologic therapy) of each kinetic parameter was related to the therapeutic outcome.

Therapeutic Outcome

Patients were followed up at 6, 12, 18, 24, and 30 weeks, with redetermination of CDAI. Data for determining the CDAI score were collected for 7 days by means of a diary card that was completed by the patient on a daily basis. Endoscopy, including examination of the large bowel and the distal part of the small bowel, was performed at least once from 10–12 weeks (mean, 11.2 weeks) after beginning pharmacologic therapy to determine the final classification of each patient as a responder or nonresponder.

Endoscopy, including examination of the large bowel and terminal ileal loop, was performed by one gastroenterologist (M.S., with 30 years of experience) after the bowel was prepared with polyethylene glycol, which was administered the previous day, and the patient fasted overnight. Immediately after the procedure, an endoscopic score was assigned according to the Crohn disease endoscopic index of severity (22). Multiple deep mucosal biopsy specimens were obtained from different colonic segments and the terminal ileal loop in all patients. By using the histologic acute inflammatory score, a score (up to a maximum of 13) was determined on the basis of grades for mucosal ulceration (grade 0–3), edema (grade 0–3), and the quantity (grade 0–3) and depth (grade 0–4) of neutrophilic infiltration by one of many

Figure 2

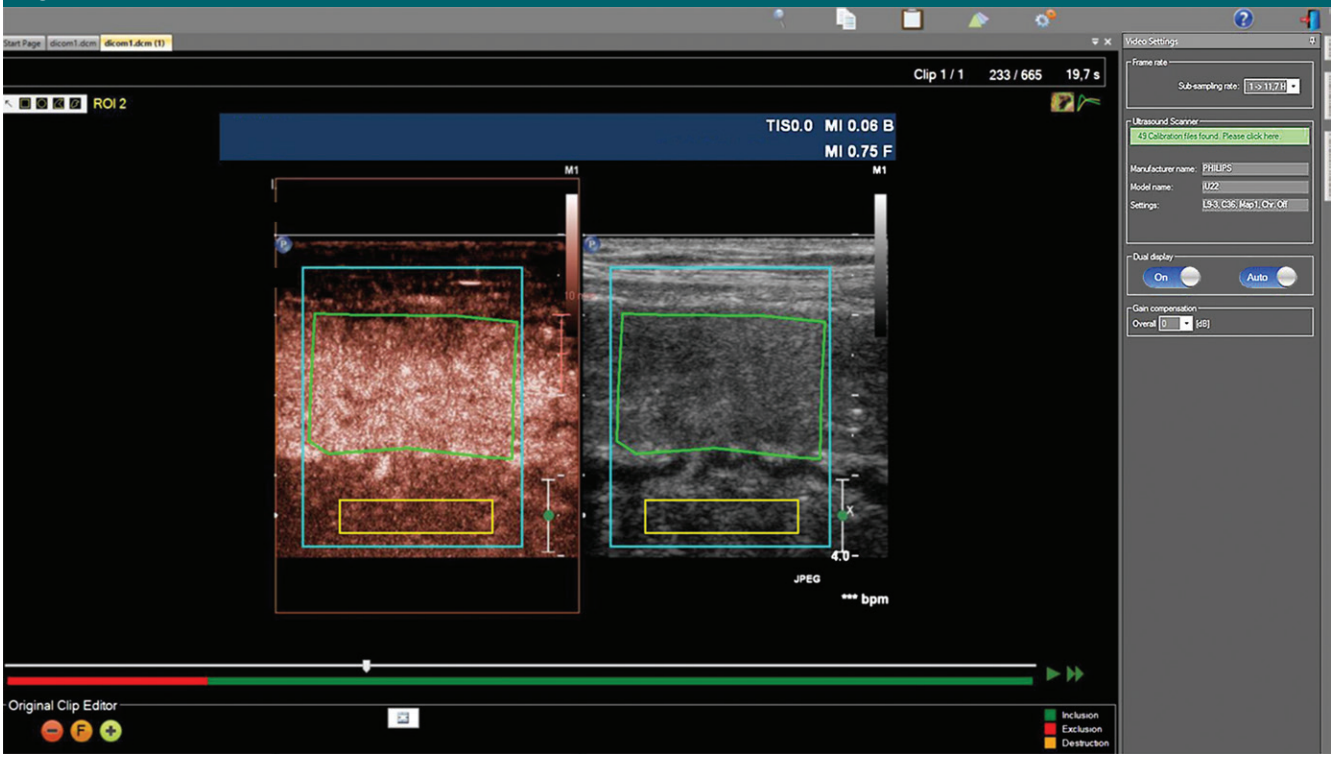

Figure 2: US images show a manually defined polygonal ROI (green) encompassing the thickened ileal tract over the anterior wall and avoiding the lumen (or encompassing the entire bowel wall if the lumen was not visible) and mesentery. Artifacts are seen along the entire extension of the terminal ileum included in the field of view on the screen. A second ROI (yellow) that served as a potential internal reference was drawn, encompassing the adjacent mesentery and excluding the ileal wall. Blue line = entire region considered for analysis and motion correction.

Figure 3

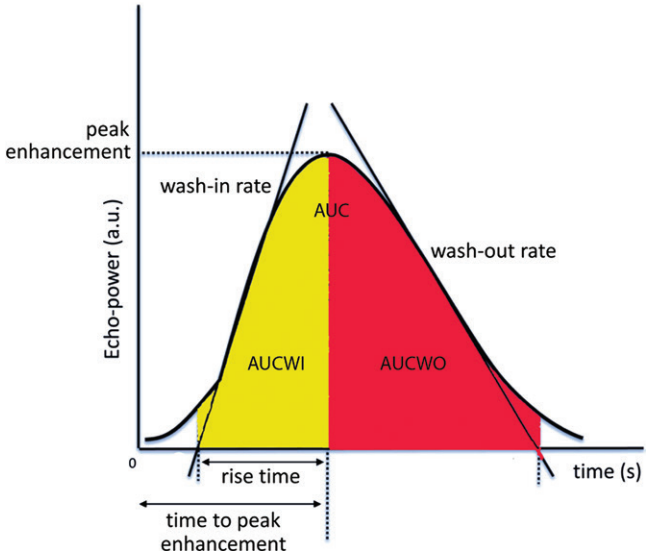

Figure 3: Graph shows a time-intensity curve. *AUC* = area under the time-intensity curve, *AUCWI* = AUC during wash-in, *AUCWO* = AUC during washout.

pathologists, each with 10–20 years of experience (23).

A patient was classified as being a responder if the Crohn disease endoscopic index of severity score decreased from 25–44 before treatment to 10–15 after treatment on the basis of ileumcolonoscopy performed 10–12 weeks after beginning pharmacologic therapy and the inflammatory score was lower than 7 and/or the CDAI score decreased by 70 or more compared with baseline; all other patients were classified as being nonresponders (24). In patients who were classified as responders, pharmacologic treatment was either maintained or increased depending on whether an increase in the CDAI was identified in the following weeks. In nonresponders, either the drug or drug combination was changed or treatment was intensified by increasing the drug dosage.

Table 2

Percent Change of the Different Kinetic Parameters in Responders and Nonresponders

	Responders		Nonresponders		
Parameter	Mean ± SD	95% CI	Mean ± SD	95% CI	*P* Value
Peak enhancement	−40.78 ± 62.85	−63.83, −17.72	53.21 ± 72.5	18.26, 88.15	.0001
Rise time	5.09 ± 49.13	−12.92, 23.12	6.24 ± 48.06	−16.92, 29.41	.93
Time to peak enhancement	8.82 ± 54.5	−11.16, 28.81	10.21 ± 43.25	−10.63, 31.06	.3
Wash-in rate	−34.8 ± 67.72	−59.64, −9.95	89.44 ± 145.32	19.39, 159.48	.001
Washout rate	−5.64 ± 130.71	−53.59, 42.3	166.83 ± 204.44	68.29, 265.37	.002
Wash-in perfusion index	−42.29 ± 59.21	−76.42, −46.72	50.96 ± 71.13	16.68, 85.25	.001
AUC	−46.17 ± 48.42	−63.93, −28.41	41.78 ± 87.64	−0.45, 84.02	.001
AUC during wash-in	−43.93 ± 54.29	−63.86, 24.03	39.79 ± 70.85	5.64, 73.94	.001
AUC during washout	−49.36 ± 47.42	−66.75, −31.97	42.65 ± 97.09	−4.14, 89.45	.001

Note.—Average values of percent change = Post – Pre × 100/Pre, where Pre is the linear value before pharmacologic treatment and Post is the linear value 6 weeks after beginning pharmacologic treatment. CI = confidence interval, SD = standard deviation.

Statistical Analysis

Statistical analysis was performed with MedCalc, version 12.2.1 (MedCalc software, Mariakerke, Belgium). The minimal appropriate patient number (n = 45) to be included in the study was estimated by considering a statistical power of 0.8, a significance criterion of 0.05, and a minimum expected difference between responders and nonresponders in each percentage of 30%. Standard deviation was considered for calculating patient sample size and was around a percentage variation of 50% on the basis of preliminary studies (16–18).

Descriptive statistics, including continuous variables, such as the age of male and female patients, body mass index, and disease duration in responders and nonresponders, were compared with Mann-Whitney U test for independent samples. Categoric variables, such as the percentage of stricturing and penetrating disease, disease location, and type of medication in responders and nonresponders, were compared with χ^2 test.

The percent change of the different kinetic parameters measured in responders and nonresponders was compared with Mann-Whitney U test for independent samples. Separate univariate logistic regressions were conducted to determine the relationship between the percent change of each kinetic parameter (independent variables) and the therapeutic outcome as responder (dependent outcome variable). Variables with a P value of less than .05 at univariate analysis were simultaneously entered into multivariate logistic regression analysis to identify kinetic parameters that were potential independent predictors of outcome.

The nonparametric Spearman sign rank correlation coefficient (ρ) was used to assess the strength of relationships between the CDAI, kinetic parameters and endoscopic grading, and the kinetic parameters measured in the terminal loop and adjacent mesentery. Interreader agreement was assessed with intraclass correlation coefficient.

Receiver operating characteristic curves were constructed for the kinetic parameters that revealed a significant difference between responders and nonresponders to determine the optimum cut-off values in accordance with the method of DeLong et al (25). The method used to identify the better cut-off value corresponds to the measurement of the maximum vertical distance of the receiver operating characteristic curve from the point (x, y) on a diagonal line (the chance line) by maximizing sensitivity plus specificity across various cut-off points. For all tests, $P < .05$ was considered to indicate a significant difference.

Results

The mean absolute extension of the small bowel segments involved in Crohn disease was 5 cm (range, 3–10 cm), with a mean depth of 2.5 cm (range, 2–4 cm) from the skin surface. Among responders (n = 31), eight patients underwent dose escalation of the biologic drug because of a moderate increase in the CDAI (10–20 units) after 12 weeks, while the remaining 23 patients maintained the drug dose; among nonresponders (n = 19), five patients underwent dose escalation of the biologic drug, four underwent azathioprine therapy, and 10 underwent surgical intervention.

Table 2 shows the results of quantitative analysis as the percent change of the different kinetic parameters in responders and nonresponders (Figs 4, 5). The quality of fit (expressed as a percentage) between the echo power signal and the theoretical lognormal curve before and after treatment was 93.76 ± 4.82 and 88.93 ± 8.33, respectively, in responders and 96.26 ± 2.32 and 82.47 ± 29.29, respectively, in nonresponders. Responders (n = 31) and nonresponders (n = 19) differed in the percent change of peak enhancement, wash-in and washout rates, wash-in perfusion index, AUC, AUC during wash-in, and AUC during washout (Fig 6). Responders and nonresponders did not

Figure 4

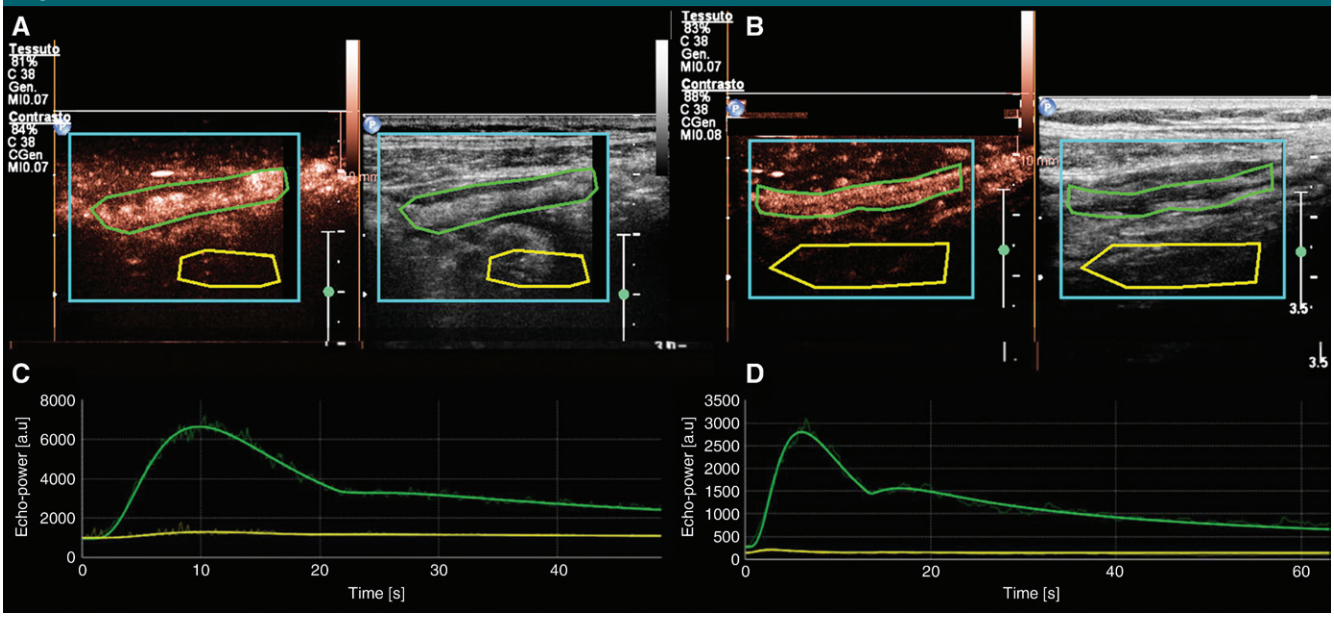

Figure 4: Difference in the time-intensity curve profile of a 25-year-old man with Crohn disease who responded to infliximab therapy. Contrast-enhanced US images obtained, *A,* before and, *B,* 6 weeks after beginning pharmacologic therapy show the terminal ileal loop. ROIs were manually drawn over the anterior bowel wall (green) and adjacent mesentery (yellow). Corresponding time-intensity curves obtained after echo power quantitation by placing an ROI over the bowel wall (green) and in the adjacent mesentery (yellow), *C,* before and, *D,* 6 weeks after beginning pharmacologic therapy show the percent variation of AUC (−37.47), AUC during wash-in (−67.2), and AUC during washout (−52.98) in the mural wall. Blue line = entire region considered for analysis and motion correction.

Figure 5

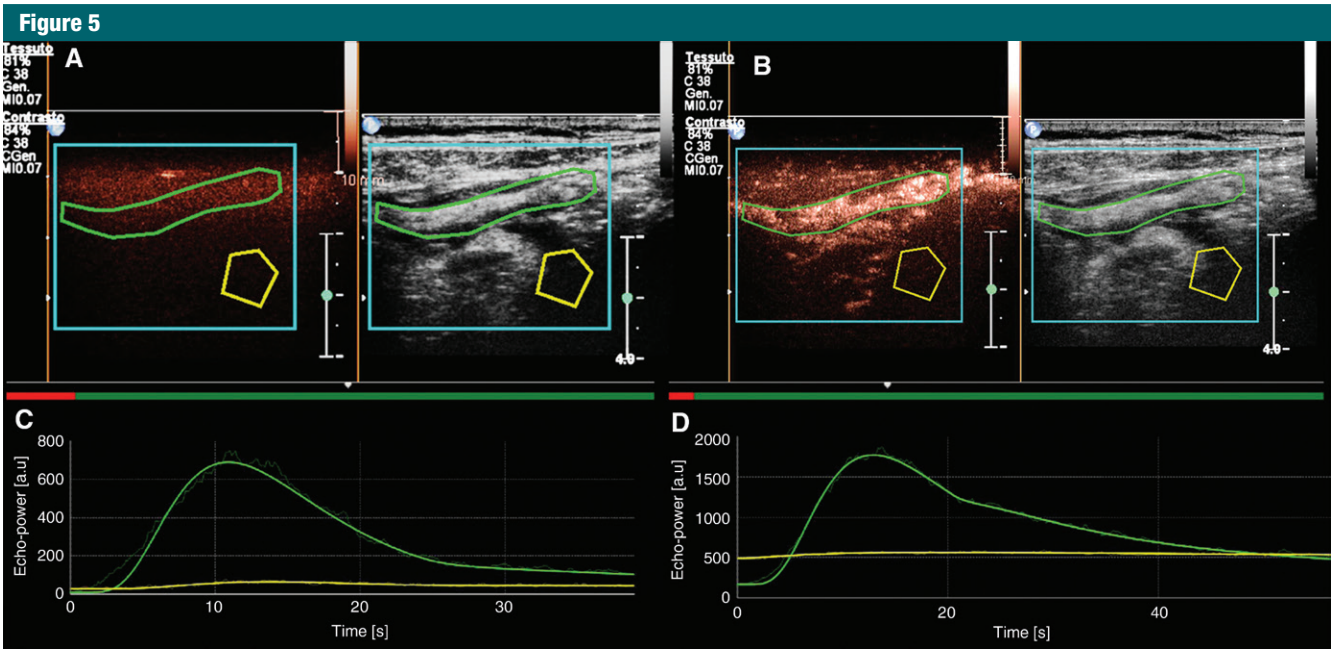

Figure 5: Difference in the time-intensity curve profile of a 45-year-old man with Crohn disease who did not respond to infliximab therapy. Contrast-enhanced US images obtained, *A,* before and, *B,* 6 weeks after beginning pharmacologic therapy show the terminal ileal loop. ROIs were manually drawn over the anterior bowel wall (green) and adjacent mesentery (yellow). Corresponding time-intensity curves obtained after echo power quantitation, *C,* before and, *D,* 6 weeks after beginning infliximab therapy show the percent variation of AUC (233.98), AUC during wash-in (190.88), and AUC during washout (256.95) in the mural wall. Persistent activity is also seen in the adjacent mesentery (yellow curve) in *D*. Blue line = entire region considered for analysis and motion correction.

Figure 6

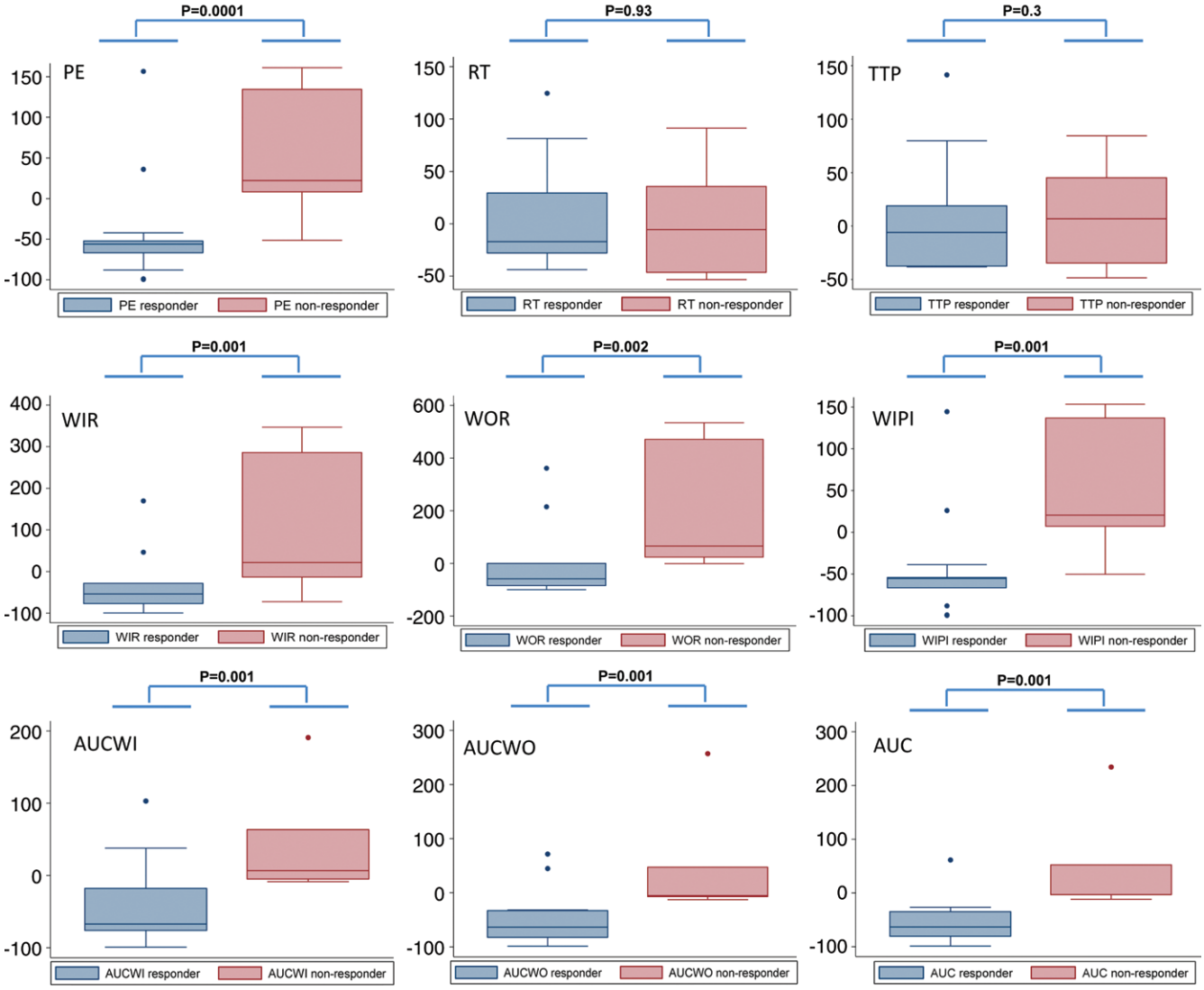

Figure 6: Box plots show the percent change of different kinetic parameters in responders and nonresponders. The top and bottom of the boxes are the first and third quartiles, respectively. The length of the box represents the interquartile range, including 50% of the values. The line through the middle of each box represents the median. The error shows the minimum and maximum values (range). An outside value (separate point outliers) is defined as a value that is smaller than the lower quartile minus 1.5 times the interquartile range or that is larger than the upper quartile plus 1.5 times the interquartile range. The *P* values for each comparison are shown over the box plots. AUCWI = AUC during wash-in, AUCWO = AUC during washout, *PE* = peak enhancement, *RT* = rise time, *TTP* = time to peak enhancement, *WIPI* = wash-in perfusion index, *WIR* = wash-in rate, *WOR* = washout rate.

differ in the percent change of rise time and time to peak enhancement.

The percent change of the different kinetic parameters within the internal reference, which corresponded to the adjacent mesentery, ranged from −1.65 to 93 in responders and from −62.39 to 407.67 in nonresponders. The nonparametric Spearman correlation coefficient between the kinetic parameters measured in the terminal loop and adjacent mesentery ranged from 0.113 to 0.42 (P = .21). The Spearman correlation coefficient between the CDAI and endoscopy ranged from 0.31 to 0.45 (P = .29). The AUC was related to both endoscopic grading (ρ = 0.77, P = .03) and CDAI score (ρ = 0.42, P = .04).

Neither rise time nor time to peak enhancement was found to predict therapeutic outcome at univariate analysis, whereas all other kinetic parameters were found to predict therapeutic outcome. No kinetic parameter was found to be an independent predictor of therapeutic outcome at multivariate logistic regression (P = .058 to P = 0.76).

Table 3

Areas under the Receiver Operating Characteristic Curve, Sensitivity, and Specificity of Percent Change to Differentiate Responders from Nonresponders

Parameter	AUC	Cut-off Value	Sensitivity (%)	Specificity (%)
Peak enhancement	0.893 (0.77, 0.96)	≤ −52.24	77.8 (60.8, 89.9)	100 (76.8, 100)
Wash-in rate	0.829 (0.69, 0.92)	≤ −28.23	86.1 (70.5, 95.3)	85.7 (57.2, 98.2)
Washout rate	0.889 (0.76, 0.96)	≤ −34.12	77.78 (60.8, 89.9)	100 (76.8, 100)
Wash-in perfusion index	0.893 (0.77, 0.96)	≤ −53.91	77.8 (60.8, 89.9)	100 (76.8, 100)
AUC	0.881 (0.75, 0.95)	≤ −26.49	86.1 (70.5, 95.3)	100 (76.8, 100)
AUC during wash-in	0.868 (0.64, 0.97)	≤ −17.69	77.8 (60.8, 89.9)	100 (76.8, 100)
AUC during washout	0.889 (0.76, 0.96)	≤ −31.72	86.1 (70.5, 95.3)	100 (76.8, 100)

Note.—Data in parentheses are 95% confidence intervals. The areas under the receiver operating characteristic curve are produced by the different cut-off values of the percent increase of AUC, AUC during wash-in, and AUC during washout.

The intraclass correlation coefficient between the quantification of the two readers ranged from 0.6 to 0.8. The intraclass correlation coefficient between the reference reviewer and the independent readers with 5 and 10 years of experience was 0.6–0.7 and 0.6–0.9, respectively. Table 3 shows that receiver operating characteristic curve analysis for identifying the optimum cut-off value for peak enhancement, AUC, AUC during wash-in, and AUC during washout had high sensitivity (77.8%–86.1%) and specificity (85.7%–100%) for differentiating responders from nonresponders among patients with Crohn disease.

Discussion

In this prospective study, we found that, among patients with Crohn disease, early assessment (6 weeks after beginning therapy) of the percent change of some kinetic parameters derived from time-intensity curve data obtained after injection of sulfur hexafluoride-filled microbubble contrast material may be used to differentiate responders to pharmacologic therapy from nonresponders. We observed a very high quality of fit between the echo power signal and the theoretical lognormal curve both in responders and nonresponders, which ensured reliable quantitative analysis.

According to our results, the assessment of bowel wall perfusion with contrast-enhanced US after 6 weeks of pharmacologic therapy may represent a reliable imaging technique for early assessment of Crohn disease in patients undergoing specific pharmacologic treatment to identify which patients have a chance to respond to ongoing pharmacologic treatment, change the pharmacologic treatment at an earlier date in nonresponders, and avoid inappropriate prolonged pharmacologic treatments that are expensive and may be toxic. Even though CT and MR enterography still represent the reference imaging techniques for grading Crohn disease activity, contrast-enhanced US may represent an alternative modality and, compared with the other modalities, offers several advantages, including low cost, portability, availability, lack of restrictions in performing frequent serial examinations at short intervals, and an absence of radiation exposure (12–18,26,27).

Previous studies showed the capabilities of contrast-enhanced US for classifying Crohn disease activity and differentiating inflammatory from fibrotic bowel lumen strictures (15–17,28–31). In previous studies, patients underwent contrast-enhanced US 12 weeks or more after beginning pharmacologic therapy, whereas the earlier assessment proposed in this study could avoid prolonged expensive and ineffective treatments in patients who have no chance to respond to treatment (15–17). In these previous studies, AUC was the main kinetic parameter that showed a difference between responders and nonresponders, whereas we found that other kinetic parameters (ie, peak enhancement, AUC during wash-in, and AUC during washout) may be used to differentiate responders from nonresponders (15–17,28–31). In our study, we calculated the percent change of each kinetic parameter for each patient. Calculating the intrapatient percent change for the different kinetic parameters limits variability and could allow a more reproducible comparison of the results between responders and nonresponders, which compensates for the extremely wide range of possible absolute values of the different parameters. Moreover, in this study, we included a larger patient population and identified thresholds for each kinetic parameter to differentiate responders from nonresponders among patients with Crohn disease with high sensitivity and specificity. We also found that internal reference that corresponds to the adjacent mesentery is unreliable for data normalization, since the percent variation of the different kinetic parameters has a low correlation with the percent variation in the bowel wall.

According to our results, echo power quantitative analysis should be used to differentiate responders from nonresponders among patients with Crohn disease, since it allows assessment of mural enhancement. We suggest that quantitative analysis of contrast-enhanced US cine clips should be routinely used in clinical practice to assess disease activity, as shown in our

and previous studies (19,33). However, it should be recognized that the quality of contrast-enhanced US cine clips depends on the experience level of the examining health care provider, the resolution of the US unit, and the location and severity of Crohn disease, with limitations in examining obese patients and the proximal ileum and jejunum.

Our study had other limitations, as well. Being an observational study, there was variability of pharmacologic treatment, including the use of corticosteroids. Our exclusion criteria could have led to biased results. Contrast-enhanced US was performed only in the terminal ileal loop; other ileal segments may have been more or less affected with a more evident or absent response. Another limitation is that we used our own data to set the thresholds that were tested for sensitivity and specificity, which may have overestimated our results. Finally, calculations of contrast-enhanced US data were performed in arbitrary units from a single machine and are not necessarily interchangeable with those from other US equipment. In conclusion, the analysis of time-intensity curves obtained after injection of microbubble contrast material 6 weeks after beginning pharmacologic treatment can be used to differentiate responders from nonresponders among patients with clinically active Crohn disease.

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