



Usefulness of abdominal ultrasonography in the analysis of endoscopic activity in patients with Crohn's disease: Changes following treatment with immunomodulators and/or anti-TNF antibodies

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KEYWORDS

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Abstract

Objective: The objective of this study was to analyze the accuracy of abdominal ultrasonography (AUS) in the assessment of mucosal healing in patients with Crohn's disease (CD) receiving immunomodulators and/or biological treatment, with ileocolonoscopy as the reference standard.

Materials and Methods: Thirty patients were included in a prospective longitudinal study. All patients underwent ileocolonoscopy and AUS before and after a minimum of one year of treatment. The Crohn's Disease Endoscopic Inflammatory Index of Severity (CDEIS) was used for endoscopic assessment whereas AUS was analyzed by means of bowel wall thickness, color Doppler grade and percentage of increase of parietal enhancement after contrast injection.

Results: In the segmental analysis, endoscopic healing was found in 71.2% of the segments and AUS findings were normalized in 62.8%, with a significant correlation between the two techniques ($\kappa = 0.76$, $p < 0.001$). In the overall assessment performed after treatment, 18 (60%) patients exhibited endoscopic remission (CDEIS < 6 points); of these patients, 15 (83.3%) had normalized sonographic

Abbreviations: AUS, abdominal ultrasonography; CDEIS, Crohn's Disease Endoscopic Inflammatory Index of Severity; CD, Crohn's disease; CEUS, contrast-enhanced ultrasonography; CI, confidence interval; CRP, serum C-reactive protein; CT, computed tomography; MRI, magnetic resonance imaging; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; SE, sensitivity; SP, specificity; SPSS, Statistical Package for Social Sciences.

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findings, with a good correlation between endoscopic remission and sonographic normalization ($\kappa = 0.73$, $p < 0.001$). Of the three variables assessed by AUS, parietal thickness was the best variable to predict mucosal healing in both analyses, segmental and global.

Conclusion: Abdominal ultrasonography is a useful and reliable technique for the assessment of the endoscopic response to treatment with immunomodulators and/or biological drugs in Crohn's disease. AUS is a highly accurate technique for evaluating the healing of the intestinal mucosa.

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1. Introduction

The introduction of immunomodulators and biological drugs on the Crohn's disease (CD) treatment scene has signified an important change in terms of the disease's treatment objectives. Simple clinical improvement, which for many years was considered the primary therapeutic goal, has given way to more ambitious objectives, most notably the induction of the disappearance of intestinal mucosal lesions. This mucosal healing, usually defined as the resolution of intestinal ulcerations,¹ needs to be assessed by ileocolonoscopy.^{2,3}

Mucosal healing has a significant clinical relevance, since current evidence suggests that patients in whom this is achieved exhibit less inflammatory activity, decreased need for corticosteroids, as well as fewer hospitalizations and surgeries.^{1,4,5}

In clinical practice, this therapeutic target has a serious drawback linked to the need for endoscopic examination to assess mucosal healing, given that it is an invasive examination that requires uncomfortable preparation and is not without its risks, which are more common in patients with inflammatory bowel disease.^{6,7} Moreover, colonoscopy is technically more challenging in subjects who have undergone abdominal surgeries, an eventuality which is often present in the CD patient population as a result of the natural course of the disease.^{8,9}

Cross-sectional imaging techniques have been used successfully in the assessment of multiple aspects of CD; moreover, compared to endoscopy, these techniques have the advantage of not being invasive.¹⁰ Although computed tomography (CT) is widely used in the imaging work-up for Crohn's disease, it carries a high radiation burden, so it cannot be used repeatedly. It is preferable to use a non-ionizing modality such as magnetic resonance (MR) imaging or abdominal ultrasonography (AUS) for follow-up evaluations. Magnetic resonance imaging (MRI) has the disadvantages of requiring specific preparation for the proper study of the small intestine and colon, as well as having a high cost and limited availability.

Abdominal ultrasound (AUS), both B-mode and contrast-enhanced US, is a technique which has already proven its usefulness in the diagnosis of CD¹¹ having the advantages over the other cross-sectional imaging techniques of not using radiation, being easily accessible and having a lower cost.^{10–12} The AUS has also proven effective in assessing endoscopic lesions associated with CD and their severity.^{13–17} However, its usefulness in the assessment of post-therapeutic endoscopic changes in CD has only been evaluated in a previous study.¹⁸

The objective of this study was to analyze the accuracy of the AUS, including contrast-enhanced US (CEUS), in the assessment of mucosal healing in patients with CD receiving immunomodulators and/or biological treatment, using ileocolonoscopy as the reference standard.

2. Materials and methods

2.1. Patients and study design

The study was conducted among patients with CD (in all patients, the diagnosis of CD was based on the Lennard–Jones criteria¹⁹) treated in the Department of Gastrointestinal Medicine of our hospital between March 2008 and December 2011. Since January 2008, information has been prospectively collected (at baseline and during follow-up) from patients with CD who had begun treatment with immunomodulators and/or biological drugs. Patients were included consecutively as long as they met the inclusion criteria, both recently diagnosed patients and patients with known Crohn's disease. We evaluated epidemiological characteristics, CD phenotype, CD Activity Index (CDAI), laboratory values, including serum C-reactive protein (CRP) levels determined by immunonephelometry (Dade Behring, Marburg, Germany; normal value ≤ 10 mg/L), results of baseline and follow-up endoscopies and of the imaging techniques. Routine clinical practice checks were performed during clinical visits scheduled every 3 months.

Patients were only included in the study if they met the following criteria: being older than 17 years of age, indication to begin immunomodulators and/or biological treatment,²⁰ and maintenance of treatment response for at least one year without receiving any other treatment during that time. Patients who declined to participate in the study and pregnant women were excluded from the study.

As is common practice in our Department, all patients underwent an ileocolonoscopy two weeks prior to the start of immunomodulators or biological therapy and AUS two weeks before treatment initiation. Both exploratory procedures were repeated after a minimum of one year from the start of treatment. In all cases, ileocolonoscopy and AUS were performed at a maximum interval of 7 days.

The approval of our center's Ethics Committee was obtained in order to conduct of the study. Prior to their inclusion in the study, patients were informed of its nature and gave their written consent.

2.2. Endoscopic protocol

All ileocolonoscopies were performed under sedation controlled by an anesthesiologist after the use of a polyethylene glycol electrolyte solution. The colonoscope used was a Pentax EC-380 LKP 4.2. All colonoscopies were performed by only one endoscopist who was unaware of the AUS findings.

The endoscopic findings were evaluated by calculating the Crohn's Disease Endoscopic Index of Severity (CDEIS).²¹ The CDEIS evaluates the severity of endoscopic lesions in CD, obtaining a score based on the division of the bowel explored by ileocolonoscopy into segments (rectum, sigmoid and left colon, transverse colon, right colon and ileum) and the presence of four findings in each segment (deep ulcers, superficial ulcers, surface of the segment affected by disease, surface of the segment affected by ulcers) and stenosis (ulcerated or non-ulcerated). The CDEIS score covers a range of values between 0 and 44 points, where a higher score indicates a greater severity of disease activity.

The findings of the ileocolonoscopies were assessed both separately for each of the six intestinal segments explored (rectum, sigmoid colon, descending colon, transverse colon, ascending colon–cecum and ileum) and globally, for the whole of the bowel explored. In the second endoscopy performed after at least one year of treatment, mucosal healing was considered to have occurred when ulcers had disappeared in the different intestinal segments explored. In the overall assessment, the treatment was considered to have induced endoscopic remission from the disease when the CDEIS score was less than 6 points.²²

2.3. Ultrasonographic protocol

US examinations were performed by using a US unit (Aplio 80; Toshiba, Tokyo, Japan), initially employing a 3–6 MHz convex-array transducer and then a 6–10 MHz probe for a detailed examination.

Each patient underwent AUS specifically for the intestine, beginning with an initial gray-scale. Bowel wall vascularity determined by color Doppler US with a special preset optimized for slow flow detection was then evaluated. The intensity of the color Doppler flow was subjectively graded as absent (grade 0), barely-visible vascularity (grade 1), moderate vascularity (grade 2) and marked vascularity (grade 3).²³

CEUS was performed with a 3–4 MHz convex probe in wideband harmonic contrast mode (pulse inversion – Toshiba Aplio) at low MI (MI < 0.10). The second generation echosignal enhancer SonoVue® (Bracco, Milan, Italy) was injected as a bolus in units of 1.2 mL, followed immediately by injection of 10 mL of normal saline solution (0.9% NaCl). To assess the vascularization of the involved bowel loop, the contrast uptake was measured over a period of 40 s by means of quantitative analysis of the brightness in regions of interest (ROIs) located in the intestinal wall using the software installed in the Aplio 80 system. Quantitative analysis of the enhancement after contrast agent injection was calculated by the percentage of increase in wall brightness by using the following formula: $[(\text{brightness post-contrast} - \text{brightness pre-contrast}) \times 100] / \text{brightness pre-contrast}$, and this was used for data analysis.¹³

Patients were examined following an overnight fasting period, with no special bowel preparation. A radiologist with 15 years of experience in ultrasonography of intestinal bowel diseases (experience exceeding >3000 examinations of the bowel) and 5 years of experience in CEUS performed all the examinations. The radiologist was unaware of the patient's endoscopic findings.

The ultrasound examination assessed: 1) Thickness of the wall of the affected segment; 2) Vascularization of the wall by

color Doppler US, and 3) Percentage of increase of enhancement in wall brightness after contrast agent injection. Abnormal parietal thickness was >3 mm (positive AUS was defined as the presence of concentric and regular increased BWT >3 mm).¹²

In the second scan, ultrasound remission was considered to have occurred when intestinal wall thickness was ≤3 mm, color Doppler flow in the intestinal wall was 0 or 1, and the percentage of parietal enhancement increase was less than 46%.^{13,24}

2.4. Statistical analysis

Basic descriptive statistics were used (absolute frequency, percentages, median and range). The correlation between the ultrasound and endoscopic variables used to estimate intestinal wall involvement before and after treatment was analyzed using the Kappa (κ) index. The ability of ultrasonography to assess endoscopic mucosal healing was determined by calculating sensitivity (SE), specificity (SP), positive and negative predictive values (PPV and NPV), accuracy and odds ratio (OR); the 95% confidence interval (95% CI) was calculated for every single value. We also calculated the areas under the ROC curves and their respective 95% CI.

The differences between the number of affected segments, the segments with ulcers and the Doppler flow before and after treatment were analyzed using the Wilcoxon test. The assessment of treatment-induced changes using the CDEIS, intestinal wall thickness and parietal contrast enhancement was analyzed by means of the McNemar test.

The determination of the best predictor variables of endoscopic mucosal healing was achieved using a univariate binary logistic regression analysis, followed by a forward and stepwise multivariate binary logistic regression analysis.

We used the Statistical Package for Social Sciences (SPSS) version 15.0.1 to describe and analyze the data, considering $p < 0.05$ as significant values.

3. Results

During the study period, thirty patients whose clinical and demographic characteristics are shown in Table 1 met the requisites. Across the 30 patients included in the study, a total of 178 bowel segments were available for ileocolonoscopy and ultrasonographic assessments, as two patients had previously undergone surgical resection of the ileum, cecum and ascending colon as a result of their illness.

Endoscopic and ultrasonographic post-treatment studies were performed after a median duration of 14 months (range: 13–25 months) counting from the time of administration of the first doses of the immunomodulators and/or biological drugs. At the time these studies were performed, 25 (83.3%) patients were in clinical remission (CDAI <150 points) while the remaining 5 (16.7%) patients exhibited clinical improvement evidenced by a decrease in the CDAI score of more than 100 points. Moreover, the median serum CRP concentration after the treatment period was 1 mg/L (range: 1–50 mg/L); in all patients who achieved clinical remission, CRP values were below 10 mg/L, while among those who exhibited clinical improvement, CRP was greater than 10 mg/L in only one patient.

Table 1 Clinical and demographic characteristics of the 30 patients with Crohn's disease included in the study.

Variables	N (%)
Sex	
Men	14 (46.7)
Disease location	
Ileum	7 (23.3)
Colon	6 (20.0)
Ileum and colon	17 (56.7)
Behavior of the disease	
Inflammatory	24 (80.0)
Fistulising	6 (20.0)
Age at diagnosis	
<16 years old	1 (3.3)
17–40 years old	20 (66.7)
>40 years old	9 (30.0)
Perianal disease	2 (6.7)
Previous surgery	16 (53.3)
Treatment	
Azathioprine	7 (23.3)
Infliximab	4 (13.3)
Adalimumab	10 (33.3)
Mercaptopurine and Infliximab	1 (3.3)
Azathioprine and Adalimumab	5 (16.7)
Azathioprine and Infliximab	3 (10.0)
CDAI score before treatment [median and range]	300 (241–360)
Serum CRP concentration (mg/L) before treatment [median and range]	12.5 (7.3–53.3)

CDAI: Crohn's Disease Activity Index; CRP: C-reactive protein.

3.1. Endoscopic findings

The ileocolonoscopies performed before and after treatment in the 30 patients included in the study were carried out without complications in all cases. The initial ileocolonoscopies were completed (colon and ileum exploration) in 18 (60%) patients; in the 12 (40%) remaining patients, endoscopic examinations failed to be completed either due to the presence of stenosis that precluded the passage of the colonoscope (colonic stenosis in 3 [10%] patients and narrowing of the ileocolonic anastomosis in 5 [16.7%] patients) or the presence of severe lesions which made it necessary to suspend the examination (4 [13.3%] patients). The number of complete endoscopies increased to 23 (76.7%) patients in the post-treatment phase; of the 7 (23.3%) patients in whom the ileocolonoscopy could not be completed at this stage, persistent stenosis was the reason preventing the examination from being completed in 5

(16.6%) patients, the appearance of a new stenosis not present during the pre-treatment ileocolonoscopy was the reason in 1, and in another the ileocolonoscopy could not be completed for technical reasons.

Endoscopic findings before and after treatment are shown in Table 2. All patients had at least one segment with ulcers. Prior to treatment, 100% of the patients had a CDEIS score greater than 6 points. In the post-treatment ileocolonoscopy, 18 (60%) patients showed endoscopic remission (CDEIS <6 points) (Fig. 1).

3.2. Ultrasound findings

Ultrasonography was technically possible in all patients, both before treatment and during post-therapeutic follow-up, making it possible to evaluate all the 178 intestinal segments available for examination. Contrast agent was administered in 22 patients in the pre- and post-treatment examination.

Sonographic findings before and after treatment are shown in Table 3. All patients presented at least one abnormal segment on the initial AUS. All ultrasound variables evaluated (mural thickness, color Doppler grade, percentage of increase of parietal enhancement) showed statistically significant improvement after treatment (Fig. 2).

4. Analysis of the abdominal ultrasonography to predict endoscopic changes

The AUS showed abnormalities only in 62 out of the 77 intestinal segments involved in the ileocolonoscopy, with 15 false-negative segments (6 in the rectum, 2 in the sigmoid colon, 2 in the descending colon, 4 in the transverse colon and 1 in the ascending colon–cecum; 7 of these segments exhibited superficial ulcers and the rest erythema). Furthermore, AUS revealed abnormalities in 13 segments which could not be assessed by ileocolonoscopy. The sensitivity and specificity of the AUS in detecting CD involvement before treatment were 80.5% and 100%, respectively.

4.1. Segmental assessment

The comparative analysis by segments between endoscopy and sonography before the start of treatment showed a good correlation, especially in the more proximal intestinal segments, with κ index scores of 0.47, 0.85, 0.82, 0.80, 0.9 and 1.0 for the rectum, sigmoid colon, descending colon, transverse colon, ascending colon–cecum and ileum, respectively.

Fifty-nine segments with ulcers were evaluated with both techniques. Healing of endoscopic lesions was observed in 42 of these segments, 37 of which also exhibited normalized

Table 2 Endoscopic changes induced by treatment with immunomodulators and/or biological drugs in 30 patients with Crohn's disease.

	Pre-treatment	Post-treatment	p
No. of segments explored [n (%)]	157 (88.2)	168 (93.3)	
Segments affected [n (%)]	77 (49.0)	43 (25.6)	<0.001 ^{McN}
Segments with ulcers [n (%)]	67 (87.0)	18 (41.9)	<0.001 ^{McN}
CDEIS score [median (range)]	15.9 (7–25)	6.8 (0–25)	<0.001 ^W

Mc: McNemar test; W: Wilcoxon test.

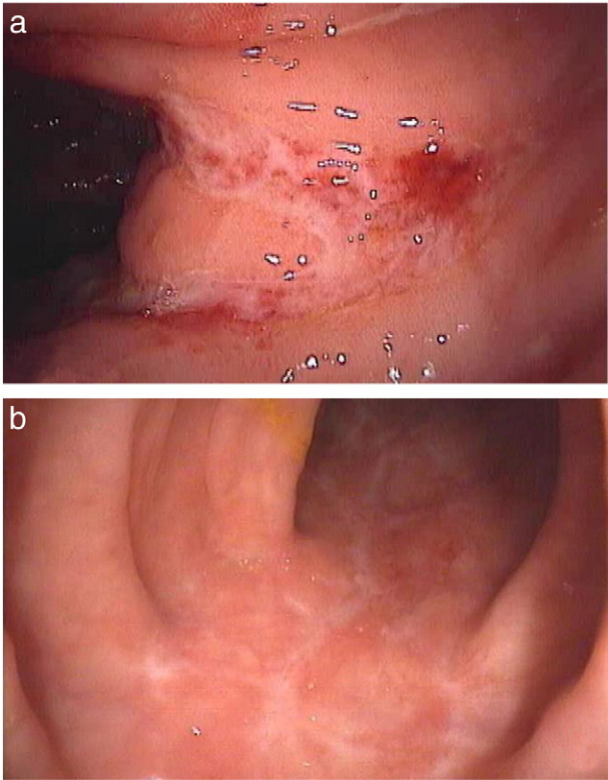


Figure 1 Endoscopic picture at baseline (a) and one year after biological treatment (b).

sonographic findings, showing a good correlation between both techniques (κ index: 0.76; $p < 0.001$). The SE, SP, PPV, NPV and OR values of the AUS for predicting endoscopic mucosal healing are shown in Table 4. The AUS had an area under the ROC curve of 0.88 (Fig. 3) and an accuracy of 89.8% (95% CI: 79.5–95.3) in predicting the presence of mucosal healing on endoscopy. The sonographic variable with the best correlation with endoscopy in the assessment of mucosal healing was the color Doppler grade of the intestinal wall ($\kappa = 0.82$, $p < 0.001$); intestinal wall thickness and parietal contrast enhancement showed a correlation with endoscopic mucosal healing of 0.76 ($p < 0.001$) and 0.67 ($p < 0.001$), respectively.

To determine the variable with the greatest ability to predict mucosal healing on colonoscopy, we performed a binary logistic regression analysis with healing as the dependent

variable (for colonoscopy No vs. Yes). A good level of correlation and a statistically significant level of prediction were obtained with all variables evaluated. We therefore performed a forward and stepwise multivariate binary logistic regression analysis which found intestinal wall thickness < 3 mm to be the variable which best helped predict endoscopic healing (92.5%).

4.2. Overall assessment

The usefulness of the AUS in the overall assessment of intestinal involvement was performed by calculating the CDEIS score. Of the 18 (60%) patients showing endoscopic remission (CDEIS < 6 points), 15 (83.3%) patients exhibited normalized sonographic findings. The correlation between endoscopic remission and a normal ultrasound was good ($\kappa = 0.73$, $p < 0.001$). The SE, SP, PPV, NPV and OR values of the abdominal US for predicting endoscopic remission are shown in Table 5. The AUS had an area under the ROC curve of 0.87 (Fig. 4) and an accuracy of 86.4% (95% CI: 75.5–93.0) in predicting endoscopic remission.

The ultrasound variable which showed the best correlation with endoscopy for predicting endoscopic remission was intestinal wall thickness < 3 mm ($\kappa = 0.86$, $p < 0.001$); intestinal wall color Doppler and parietal contrast enhancement showed a correlation with endoscopic remission of 0.85 ($p < 0.001$) and 0.76 ($p < 0.001$), respectively.

We once again performed a binary logistic regression analysis to see which variable was the best predictor of healing; the OR values for each estimator were not calculated, as these yielded unstable models due to strong collinearity. A newly performed forward and stepwise multivariate binary logistic regression analysis found that the variable with the best prognostic value was intestinal wall thickness < 3 mm (95.5%).

4.3. Correlation between mucosal healing, clinical status and the serum concentration of C-reactive protein

The correlation between mucosal healing (endoscopic remission) and clinical remission (CDAI < 150 points) was poor ($\kappa = 0.28$); 7 (23.3%) patients in clinical remission exhibited no mucosal healing. The correlation with serum CRP values was also weak ($\kappa = 0.1$); 11 (36.6%) patients with serum CRP values within the normal range (< 10 mg/L) exhibited no mucosal healing.

Table 3 Ultrasound changes induced by treatment with immunomodulators and/or biological drugs in 30 patients with Crohn's disease.

	Pre-treatment	Post-treatment	p
No. of segments assessed [n (%)]	178 (100)	178 (100)	
No. of segments affected [n (%)]	75 (42.1)	29 (16.3)	$< 0.001^{McN}$
Intestinal wall thickness [mm; median (range)]	5 (4–9)	2 (1–8)	$< 0.001^W$
Patients with color Doppler flow grade 2–3 [n (%)]	27 (76.7)	6 (20.0)	$< 0.001^{McN}$
Percentage of increase of intestinal wall enhancement [%; median (range)] (n = 22)	65 (29.0–100)	44 (0.0–100)	$< 0.001^W$
Complications			
Fistulas [n (%)]	4 (13.3)	0	

W: Wilcoxon test; McN: McNemar test.

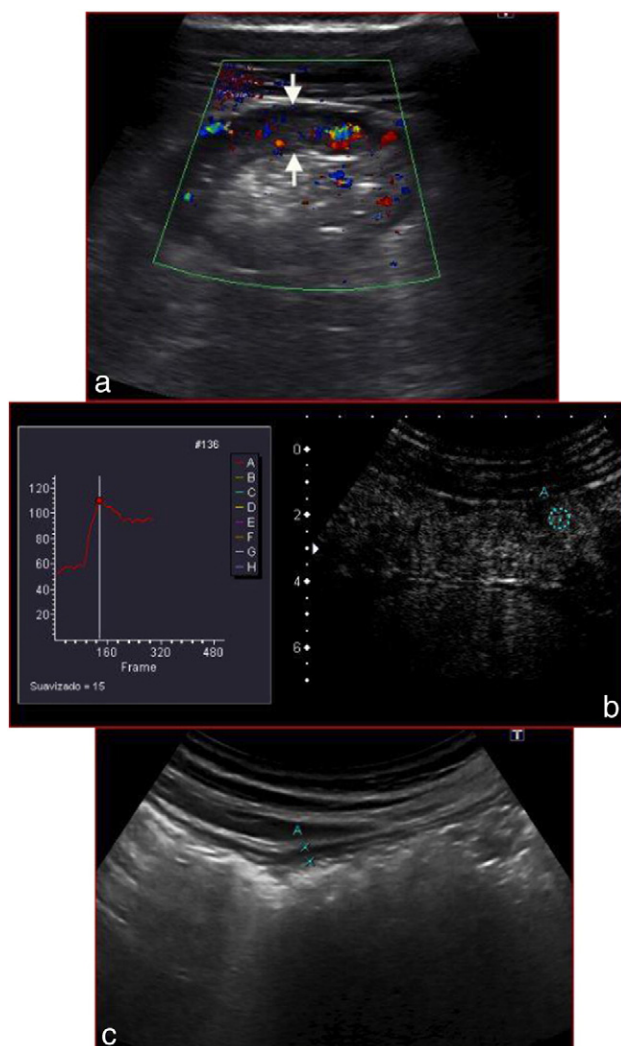


Figure 2 (a) Pre-treatment transverse US scan of the ascending colon shows marked color Doppler flow in the thickened wall (between arrows). (b) Post-contrast agent longitudinal image before treatment with the anti-TNF drug shows marked enhancement of the walls. An 82% percentage of enhancement increase in wall brightness was measured in the brightness-time curve (ROI in bowel wall). (c) Longitudinal US section of the ascending colon of the same patient depicts normal wall (<3 mm) (A) after 1 year of the treatment with anti-TNF.

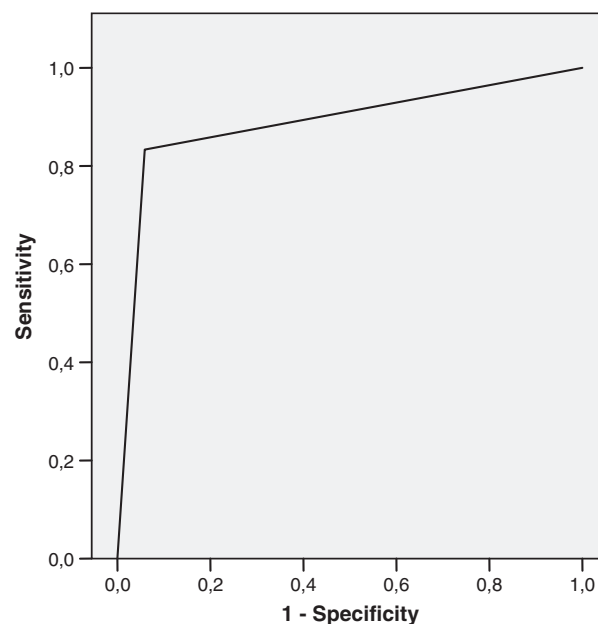


Figure 3 ROC curve for performance of abdominal ultrasound analyzed segment-by-segment for the diagnosis of endoscopic mucosal healing induced by immunomodulators and/or biological drugs in 30 patients with Crohn's disease. Mean area under the curve was 0.88.

5. Discussion

The benefits of achieving mucosal healing in CD have been assessed in several studies.^{1,4,5} It is now established that both immunomodulators and biological drugs share the ability to achieve this therapeutic goal in patients with CD.^{1,25} In clinical practice, it is important to know how long after starting the treatment \rightarrow was mucosal healing achieved, as this has important implications for disease prognosis and the therapeutic strategy to be followed. However, the precise time that patients should be evaluated to assess mucosal healing is not clearly established yet,²⁵ which is a serious drawback given that ileocolonoscopy, the diagnostic technique of choice, cannot be performed repeatedly in the same patient due to its invasive nature and the risks involved, especially in patients with inflammatory bowel disease.^{6,7}

Table 4 Results by intestinal segments for the ultrasound variables used to evaluate endoscopic mucosal healing induced by immunomodulators and/or biological drugs in 30 patients with Crohn's disease. Values are % (confidence interval of 95%).

Variables	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Odds ratio
Intestinal wall thickness <3 mm	88.1 (75.0–94.8)	94.1 (73.0–99.0)	97.4 (86.5–99.5)	76.2 (54.9–89.4)	118.4 (12.8–1096.4)
Color Doppler flow grade 0 or 1	97.6 (87.7–99.6)	82.4 (59.0–93.8)	93.2 (81.8–97.7)	93.3 (70.2–98.8)	191.33 (18.4–1992.5)
Percentage of increase of enhancement <46%	85.2 (67.5–94.1)	84.6 (57.8–95.7)	92.0 (75.0–97.8)	73.3 (48.0–89.1)	31.63 (5.1–199.7)
Thickness <3 mm + color Doppler flow grade 0–1 + enhancement <46%	83.0 (69.4–91.7)	94.1 (73.0–99.0)	97.2 (85.8–99.5)	69.6 (49.1–84.4)	80–0 (9.0–705.7)

Table 5 Results of the different ultrasound variables to assess endoscopic remission (Crohn's Disease Endoscopic Index of Severity <6 points) induced by immunomodulators and/or biological drugs in 30 patients with Crohn's disease. Values are % (confidence interval of 95%).

Variables	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Odds ratio
Intestinal wall thickness <3 mm	86.8 (65.5–95.8)	96.2 (71.7–99.6)	97.1 (77.1–99.7)	83.3 (58.4–94.7)	165 (7.3–3752)
Color Doppler flow grade 0 or 1	97.4 (79.1–99.7)	80.8 (53.7–93.8)	88.1 (68.2–96.2)	95.5 (97.9–99.5)	155.4 (6.8–3552.6)
Percentage of increase of enhancement <46%	68.2 (39.3–87.6)	96.2 (71.7–99.6)	93.8 (59.8–99.3)	78.1 (53.7–91.7)	53.6 (2.4–1187.3)
Thickness < 3 mm + color Doppler flow grade 0–1 + enhancement <46%	83.3 (60.8–94.2)	91.7 (64.6–98.5)	93.8 (71.7–98.9)	78.6 (52.4–92.4)	55 (5.02–602.2)

In our study, we analyzed whether the endoscopic changes produced by treatment with immunomodulators or biological drugs could be predicted using sonographic evaluation. The AUS allowed a highly accurate assessment (89.8% in the segmental analysis and 84.6 in the analysis by patient) of mucosal healing induced by treatment with immunomodulators and/or biological drugs. Based on our results normalization of ultrasound findings could be considered as a strong predictor of mucosal healing detected at endoscopy, so this could reduce the need for follow-up endoscopy. Thus, our results show that AUS, a non-invasive technique, is effective for the monitoring of treatment-induced intestinal morphological changes in CD, which has two important implications for routine clinical practice. First, abdominal US findings can be helpful in deciding when ileocolonoscopy should be ordered to confirm mucosal healing. Second, the AUS can be used to explore all intestinal segments,

including those not reachable with ileocolonoscopy; in fact, in our study, AUS was able to explore 100% of the intestinal segments, while only 88% could be assessed by means of the initial ileocolonoscopy.

Our results agree with a sonographic study published recently.¹⁸ The study of Castiglione et al. included 133 patients (66 patients with CD treated with biologics and 67 patients receiving thiopurines) and evaluated transmural healing by sonography. Similar to our study good agreement was found between transmural healing and mucosal healing ($k = 0.63$); all but 2 cases of transmural healing were associated with mucosal healing. However, in this study sonographers only evaluated thickness of the intestinal wall, whereas in our study we also evaluated the color Doppler grade and the enhancement of the bowel wall. On the other hand, although mucosal healing was defined similar to our study as the absence of ulceration in any segment in Castiglione's study, results were evaluated per patient, whereas in our study mucosal healing was evaluated segment-by-segment. Finally, Castiglione's article emphasizes the difference in the percentage of mucosal healing between different treatments, however our article highlights the correlation between endoscopic mucosal healing and transmural ultrasonographic healing.

In our study, the ultrasound variable which best detected mucosal healing by segments was color Doppler flow (highest sensitivity, 97.6%), and the variable that best detected the absence of healing was intestinal wall thickness (greatest specificity, 94.1%; only one patient with a thickness <3 mm did not exhibit mucosal healing). Overall, the US variable which proved to be the best for predicting mucosal healing was the presence of an intestinal wall thickness of less than 3 mm, correlating well with mucosal healing both in the segmental analysis and the overall analysis based on the CDEIS score. In the multivariate analysis, wall thickness also proved superior to the other US variables evaluated (color Doppler grade flow and parietal enhancement after contrast administration). On the basis of our results, endoscopic treatment response can be securely assessed with B-mode ultrasound without contrast agent administration or the use of special software for interpretation. This entails a simplification of the use of ultrasonography in the clinical practice, since the assessment of wall thickness is technically easier and faster than the evaluation of other US parameters. Moreover, in our experience it is not possible, even with the newer machine, to evaluate the enhancement if the bowel wall is not clearly identified or if it is too thin (<3 mm). Moreover, motion artifacts produced by

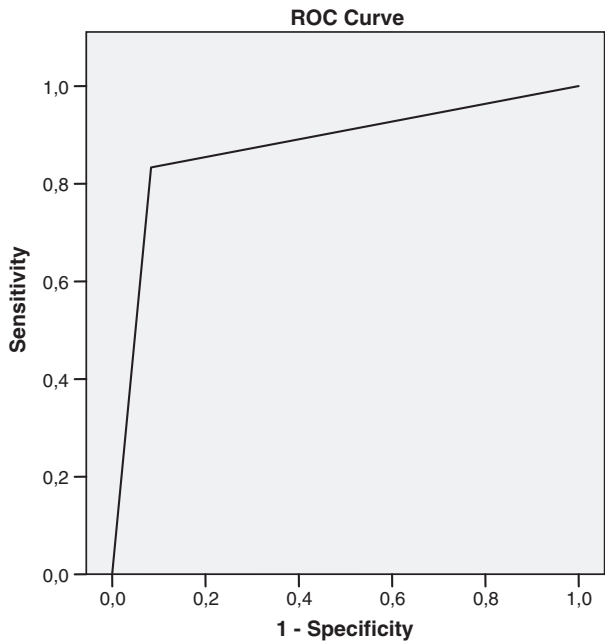


Figure 4 ROC curve for performance of the abdominal ultrasound for the diagnosis of endoscopic remission (Crohn's Disease Endoscopic Index of Severity <6 points) induced by immunomodulators and/or biological drugs in 30 patients with Crohn's disease. Mean area under the curve was 0.87.

peristalsis or intestinal contents complicate the evaluation of color Doppler grade.

According to the criteria used in previous articles, in this study the endoscopic disappearance of ulcers was considered mucosal healing and treatment-induced histological changes were not assessed.^{22,26} Some authors suggest that the absence of histological activity is required to establish the existence of deep remission of CD, as opposed to only macroscopic healing of the mucosa.²⁵ A recent study showed a good correlation between CEUS and the histological parameters of CD,²⁷ while another study appears to suggest that the AUS is correlated with the histological changes produced after treatment.²⁴ The ability of the AUS to assess histological changes remains to be analyzed and should be the focus of future studies. Enhancement of the intestinal wall yielded several false positives (patients with mucosal healing and parietal enhancement greater than 46%). This could be related to the persistence of microscopic activity and microvascularization in areas that are macroscopically normal. Transmural healing or persistent inflammation should be evaluated in studies assessing the histological response of CD in surgical specimens, by analyzing the results of the different ultrasound variables. This limitation can be applied to other cross-sectional imaging techniques (CT, MRI).

In our opinion a significant advantage of AUS over endoscopy for assessment of CD is that, excluding the rectum, the sonographic exam is always complete. Theoretically, bowel loop assessed at US might not have corresponded to the region assessed at colonoscopy. Cases of false-positive or false-negative results could arise from US measurements localized in bowel segments different from bowel segments seen at colonoscopy. As indicated above, in a significant percentage of our patients, the initial ileocolonoscopy failed to explore all intestinal segments, as it has happened in other studies which compared endoscopic findings with those obtained using cross-sectional imaging techniques in CD.²⁸ In our study, pre-therapeutic ileocolonoscopies were incomplete in 40% of patients, and although this figure was lower for post-treatment endoscopies, it still reached 23% of patients. The main reason why some endoscopies could not be completed was the presence of intestinal stenosis that could not be passed by the endoscopy, which in 62.5% of cases were caused by severe disease recurrences with surgical anastomosis stenosis and the inability to explore the neoterminal ileum. Despite stenosis being observed on endoscopy, none of the patients showed symptoms of intestinal obstruction at baseline or during follow-up, nor did the ultrasound reveal signs of obstruction in any of the patients.

The mucosal healing rates achieved in our series of patients, both in the analysis by intestinal segment (70%) as in the overall analysis using the CDEIS (60%), were higher than those reported by other authors.^{25,18} In our view, this difference can be due to our selection criteria, based on the fact that repeated colonoscopy and AUS were performed focused to patients with clinical remission and long-term maintenance therapy without receiving any other treatment. It can be an important bias that can make the real percentage of mucosal healing difficult to determine.

A limitation of our study is that the criteria that have been used to define normality for two of the US parameters (color Doppler flow and parietal contrast enhancement) are not firmly established, given that they are based on the results of case series studies,^{24,13} which may have affected

the results obtained with these two variables. However, this consideration does not affect the intestinal wall thickness variable, which was the parameter that yielded the best results in our study, because a meta-analysis¹² demonstrated its value in detecting the presence of CD. Other limitations of this study include the limited sample size and the difficulty in using AUS to assess certain segments such as the rectum. Furthermore, although the use of AUS is difficult in clinical trials, it can be used in clinical practice at least by operators with experience.

To conclude, in our experience there is a high correlation between changes detected by AUS and endoscopy after treatment with immunomodulators and/or biological drugs, so that AUS could constitute as a useful technique for the assessment of intestinal mucosal healing.

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