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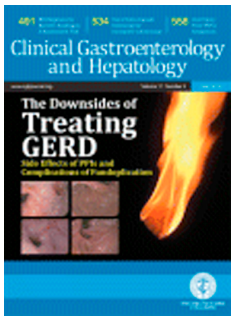

Ultrasonography tight control and monitoring in Crohn's disease during different biological therapies: a multicenter study

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Ultrasonography tight control and monitoring in Crohn's disease during different biological therapies: a multicenter study

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EC, FZ, FC, AR, GM, methodology;

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FZ and AS: formal analysis;

EC and GM: supervision.

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Abbreviations used in this paper:

BUS: bowel ultrasonography; CD: Crohn's disease; BWT: bowel wall thickening; TH: transmural healing; HBI: Harvey Bradshow Index; CRP: C-reactive protein; FCal: faecal calprotectin; s.c.: sub cutaneous; i.v.: intravenous; HR: hazard ratio.

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Abstract

Background & Aim: Bowel Ultrasonography (BUS) is a non-invasive tool for evaluating bowel activity in Crohn's disease (CD) patients. Aim of our multicenter study was to assess whether BUS helps to monitor intestinal activity improvement/resolution following different biological therapies. **Methods:** Adult CD patients were prospectively enrolled at 16 sites in Italy. Changes in BUS parameters [i.e. bowel wall thickening (BWT), lesion length, echopattern, blood flow changes and transmural healing (TH: normalization of all BUS parameters)] were analyzed at baseline and after 3, 6 and 12 months of different biological therapies. **Results:** One hundred and eighty-eight out of 201 CD patients were enrolled and analyzed (116 males [62%]; median age 36 years). Fifty-five percent of patients were treated with adalimumab, 16% with infliximab, 13% with vedolizumab and 16% with ustekinumab. TH rates at 12 months were 27.5% with an NNT of 3.6. TH at 12 months after adalimumab was 26.8%, 37% after infliximab, 27.2% after vedolizumab and 20% after ustekinumab. Mean BWT improvement from baseline was statistically significant at 3 and 12 months ($p<0.0001$). Median Harvey-Bradshaw index, C-reactive protein and fecal calprotectin decreased after 12 months from baseline ($p<0.0001$). Logistic regression analysis showed colonic lesion was associated with a higher risk of TH at 3 months and a greater BWT at baseline was associated with a lower risk of TH at 3 months [p=0.03 (OR 0.70, 95%CI 0.50-0.97)] and 12 months [p=0.01 (OR 0.58, 95%CI 0.38-0.89)]. At 3 months therapy optimization during the study was the only independent factor associated with a higher risk of no ultrasonographic response [p=0.02 (OR 3.34, 95%CI 1.18-9.47)] and at 12 months disease duration [p=0.02 (OR 3.03, 95%CI 1.15-7.94)]. **Conclusion:** Data indicate that BUS is useful to monitor biologics-induced bowel activity improvement/resolution in CD.

Key words: Crohn's disease; imaging; inflammation; bowel ultrasonography; biologicals; monitoring.

Introduction

Evidence accumulated over the past decade indicates that bowel Ultrasonography (BUS) is an accurate diagnostic tool not only in the evaluation of disease activity and complications but also for monitoring disease progression and assessment of therapeutic response in Crohn's disease (CD)[1]. CD is a transmural disease in which progressive inflammation leads to intestinal wall thickening, fibrosis, and penetrating complications. Therefore, inflammatory burden and disease prognosis may not be adequately reflected by only assessing the mucosal layer. The increasing use of BUS in CD has introduced new challenges, including how to interpret lesion changes mediated by anti-inflammatory therapies (corticosteroids, immunosuppressants and biological drugs) with different ultrasonographic techniques and how to define remission after treatments[2,3]. Some studies report data regarding the potential role of transmural healing (TH) as a long-term prognostic factor. Castiglione and colleagues showed that normalization of the parietal thickening assessed by BUS was associated with a higher rate of steroid-free clinical remission and a lower rate of relapse at one year compared to mucosal healing and no healing[4]. Zorzi and colleagues demonstrated that ultrasonographic response is noted in more than 50% of patients after one-year anti-TNF therapy and this response is associated with significantly reduced long-term risk of corticosteroid need, hospitalizations, and/or surgeries among patients with CD[5]. Monitoring CD patients with an ultrasonographic tight control during biological therapies could be a valuable method to assess lesion remodeling or healing and a decision instrument to continue or change therapies. The development of a standardized BUS imaging interpretation and reporting pattern among sonographers will improve comparability of BUS results among various centers globally, with a subsequent improvement in the quality of multicenter BUS studies and training with a wider dissemination of this technique[6]. Aim of our multicenter study was to assess changes in BUS parameters, including TH induced by different biological therapies.

Methods

Patients were prospectively enrolled at 16 sites in Italy between February 2018 and February 2019 and followed for a year. Patients were eligible if they were ≥18 years with a proven diagnosis of ileal and/or ileocolonic CD and eligible for biological therapies[7]. Diagnosis, CD location and patients' assessment were made according generally accepted recommendations[1]. Patients were followed for 12 months, with visits and BUS at baseline, 3 months, 6 months, and 12 months. At each visit, the Harvey Bradshaw Index (HBI) was determined and blood and faecal biomarkers (C-reactive protein-CRP, and faecal calprotectin-FCal) were prospectively collected and recorded. Patients with an HBI score less than 5 were considered to be in remission. Exclusion criteria included pregnancy, ileal or colonic stoma and obesity (body mass index >30). Patients with CD lesions restricted to the gastroduodenal or anorectal areas and patients with abdominal abscess were also excluded. Written informed consent was obtained from all study participants. The study was approved by the local ethics committee of the study coordinator center (number 174/17).

Biological treatments

In this prospective, multicenter study patients received standard of care according to the ECCO guidelines for therapies[7]. Induction therapy consisted of 5 mg/kg infliximab (i.v.) at weeks 0, 2, and 6 or 160 mg of adalimumab (s.c.) at week 0 and 80 mg at week 2 or 300 mg of vedolizumab (i.v.) at weeks 0, 2 and 6 or 130 mg of ustekinumab (adapted according patient weight: ≤ 55 kg 260 mg, > 55

kg to ≤ 85 kg 390 mg, > 85 kg 520 mg) (i.v.) at week 0. Maintenance therapy consisted of 5 mg/kg infliximab every 8 weeks or 40 mg of adalimumab every 2 weeks or 300 mg of vedolizumab (i.v.) every 8 weeks or 90 mg of ustekinumab (s.c.) every 8 weeks, respectively. Treatment was intensified reactively when loss of clinical response was documented in association with objective evidence of active disease assessed by increased FCal or CRP and at BUS. Intensification was considered 10 mg/kg (i.v.) every 8 weeks for IFX, 40 mg (s.c.) every week for adalimumab, 300 mg (i.v.) every 4 weeks for vedolizumab and 90 mg of ustekinumab (s.c.) every 4 weeks for ustekinumab.

BUS

BUS was performed at baseline, 3, 6, and 12 months after therapies. Patients were examined in the fasting state. The US examinations were performed by the gastroenterologists managing and treating the patient with different US devices using convex probe (1–8 MHz) and a high-frequency, linear-array transducer (3–11 MHz). Examiners were all experienced bowel sonographers and were not blinded to clinical/biochemical parameters of the patient. Disease site (based on bowel wall thickening >3 mm for ileum, and >4 mm for colon), extent of lesions, echo pattern (preserved and not preserved), presence of lymph-nodes and/or fibrofatty proliferation, presence of complications [stenosis, prestenotic dilation, abscess, fissures (lesion originating from deep ulcerations of the intestinal wall visualized as subtle hypoechoic irregularities of the bowel surface) or fistulas] were evaluated using BUS as previous described[8],[9]. As a semi-quantitative method for determining disease activity, the vascularity within the affected bowel wall areas was assessed by duplex US examination using the Limberg score[10]. The most affected bowel segment at baseline was used for all BUS parameters. The cut-off value of bowel wall thickening (BWT) and all parameters were previously defined in two meetings. The first one was conducted for standardization of all bowel parameters and to reach a consensus about lesions and the second one to share difficulties during the enrollment. Representative examples of the lesions were analyzed and discussed by participants following EFSUMB guidelines[9]. In all recruited patients, a Case Report Form was generated for BUS parameters by each operator.

BUS changes were categorized as:

a) improved lesions defined as (a) those with improvement (> 1 mm) or normalization of bowel wall thickening (BWT, normal value for small bowel < 3 mm, for large bowel < 4 mm), (b) decreased length of disease, (c) Limberg score improvement, (d) no worsening of the other disease parameters of active inflammation or fistulizing disease. All patients with improved lesions had at least 2 improved ultrasonographic parameters. Transmural healing was defined as normalization of all parameters.

b) worsened lesions defined as those with a deterioration of measurements of all parameters of active inflammation.

c) Unchanged lesions defined as those with unchanged inflammatory parameters.

Statistical analysis

The sample size was calculated based on per-patient analysis for the association of baseline patient/disease characteristics with BUS response status. For a characteristic with 50% prevalence, with a power of 80%, an alpha of 0.05, in order to detect a relative risk of 2, 150 patients were needed.

Demographic data were expressed as median and range. Differences between disease characteristics in different treatment groups were analyzed by Kruskal-Wallis test correct for multiple comparison using Dunn's correction. Changes in BUS parameters after 3, 6 and 12 months were analyzed using Wilcoxon test and paired *t* test; difference between proportion and NNT was tested by Chi-square test.

Logistic regression analyses were performed to examine the relationship between the outcomes (transmural healing and unchanged/worsened lesions) at 3 and 12 months as dependent variables and possible predictors as independent variables. The following variables were included in the univariable analysis: gender, smoking habits, age at CD diagnosis, disease location, behavior, disease site evaluated at US (ileal/colonic site), previous surgery, previous anti-TNFs exposure, disease duration, indication to therapies, type of therapies, optimization; combination therapy, concomitant therapies with steroids, positive FCal; positive CRP, HBI, all BUS parameters. The multivariable analysis adjusted for disease duration, disease location, prior anti-TNF exposure, types of therapies was performed using a multiple logistic regression model. A $p<0.05$ was considered statistically significant. In this multivariate model we included both factors/variables that had statistical weight and highlighted disease burden. The cumulative probabilities of TH and unchanged/worsened lesions for each treatment were calculated using the log-rank (Mantel-Cox) test and hazard ratio (HR) (Mantel-Haenszel) with 95% confidence interval (CI).

Results

Two hundred and one CD patients were eligible. Thirteen patients (6%) discontinued the trial prematurely (Figure 1 supplementary material). Clinical characteristics of the 188 patients analyzed are reported in Table 1. Fifty-five percent of patients was treated with adalimumab, 16% with infliximab, 13% with vedolizumab and 16% with ustekinumab. During the 12 months study, 13% of patients needed biological therapy optimization based on clinical, biochemical and/or sonographic evaluations. CD duration (p=0.01), prior surgery (p=0.002), prior anti-TNF therapies (p<0.0001), HBI at enrolment (p=0.004) were statistical different in the four groups of treatment (Table 1 supplementary material).

Ultrasonographic response

All BUS parameters at baseline visit (V0), at 3 (V1), at 6 (V2) and at 12 months (V3) were recorded and compared (Table 2). Signs of active inflammation at ileal level in terms of BWT, lesion length, preserved echo pattern, blood flow according Limberg score, fissures, lymph nodes and fibrofatty proliferation had significantly improved at each time point. The same behavior was observed at colonic level except for the echo stratification and presence of fissures (Table 2). No statistical differences for the presence of complications (stenosis, fistulae) were observed.

After 3 months of treatment, we observed a significant proportion of patients experienced complete healing of lesions compared to V0 (p<0.0001) (Table 3). At V1, TH rate was 16.4% with NNT of 6.1 (14.5% and 26.6% in patients with ileal and colonic disease, respectively). (Figure 1, panel a). A significant lesions improvement rate was observed in 36.7% of patients with NNT of 2.7 (40.5% and 16.6% in patients with ileal and colonic disease, respectively). Eighty-eight out of 188 of patients (46.8%) had unchanged/worsened lesions (Table 3) (Figure 1, panel b).

After 6 months of treatment we observed a significant proportion of patients presented TH compared with baseline (p<0.0001) (Table 3). At V2, TH rate was 24.5% with NNT of 4.1 (21.9% and

40% in patients with ileal disease and colonic disease, respectively). A significant lesions improvement rate was observed in 38% of patients with NNT of 2.6 (41% and 20% in patients with ileal and colonic disease, respectively). Sixty-four out of 171 patients (37.4%) had no lesions changes or worsened damage (Table 3).

At 12 months of treatment we observed a significant proportion of patients experienced complete healing of lesions compared to V0 ($p<0.0001$) (Table 3). At V3, TH rate was 27.6% with NNT of 3.6 (24% and 47.8% in patients with ileal and colonic disease, respectively). A significant lesions improvement rate was observed in 36% of patients with NNT of 2.9 (40.6% and 21.7% in patients with ileal and colonic disease, respectively). Fifty-four out of 156 patients (34.6%) pts had no changes or worsened damage (Table 3).

Considering different drugs, at 12 months **TH** was observed in 26.5% of patients treated with adalimumab, in 37% with infliximab, in 27.2% with vedolizumab and in 20% with ustekinumab. No statistically significant difference in speed of effectiveness of TH among therapies was observed (Figure 2, panel a), but considering the lesion improvement, patients treated with ustekinumab had a lower rate than patients treated with infliximab [HR = 0.4 (95%Cl 0.2-0.9), p=0.037] (Figure 2, panel b). Furthermore, patients treated with ustekinumab showed higher rates of **unchanged/worsened lesions** than patients treated with anti-TNFs [adalimumab vs ustekinumab: HR = 2.1 (95%Cl 1.12-3.9), p=0.02; infliximab vs ustekinumab: HR= 2.7 (95%Cl 1.9-6.4), p=0.017] (Fig 2, panel c).

At 3, 6 and 12 months after infliximab, patients experienced **TH** in 13%, 22% and 37%, respectively. Fifty-one percent, 52% and 40% of patients showed **improved lesions** while 36%, 26% and 23% of patients had **unchanged/worsened lesions**.

At 3, 6 and 12 months after adalimumab, patients experienced **TH** in 17.5%, 29% and 26.5%, respectively. Thirty-seven percent, 38% and 40% of patients showed **improved lesions** while 46% and 33% (both 6 and 12 months) of patients had **unchanged/worsened lesions**.

At 3, 6 and 12 months after vedolizumab, patients experienced **TH** in 12.5%, 23% and 27%, respectively. Thirty-seven percent, 27% and 32% of patients showed **improved lesion** while 50% (both for 3 and 6 months) and 41% of patients had **unchanged/worsened lesions**.

At 3, 6 and 12 months after ustekinumab, patients experienced **TH** in 20%, 14% and 20%. Twenty percent, 31% and 32% of patients showed **improved lesions** while 60%, 55% and 48% of patients had **unchanged/worsened of lesions** (Fig. 2, panel d).

Clinical response

After 3, 6 and 12 months a significant proportion of patients achieved clinical remission and normalization of CRP and FCal comparing to V0 (Table 3). At 12 months, patients in clinical and biochemical remission had a higher rate of TH or improved lesions than unchanged/worsened lesion group (Figure 2 supplementary material).

Optimization treatment strategy

During the study, 13% of patients (25/188) needed therapy optimization based on clinical, biochemical and/or sonographic evaluations. At V1, 3 out of 5 patients experienced an improvement of the lesions after dose escalation. At V2, 2 out of 12 improved ultrasonographic lesions and further 2

patients achieved TH. At V3, 8 patients received therapy optimization. Overall dose escalation induced lesions improvement in 41% of patients.

Prediction of risk for TH or unchanged/worsened lesions

Logistic regression was performed to examine the relationship between TH or unchanged/worsened lesions outcomes at 3 and 12 months as dependent variables and possible predictors as independent variables. Univariable analysis showed a greater BWT at baseline was associated with a lower risk of **TH** at 3 [p=0.018 (OR 0.69, 95%CI 0.5-0.94)] and 12 months [p=0.006 (OR 0.65, 95%CI 0.48-0.89)]. Colonic lesion was associated with a higher risk of **TH** at 12 months [p=0.02 (OR 3.14, 95%CI 1.14-8.65)], inversely at 12 months patients treated with ustekinumab were associated with a lower risk of **TH** [p=0.04 (OR 0.24, 95%CI 0.06-0.97)] (Table 4). At the same level, univariable analysis showed prior surgery was associated with a slight lower risk of **unchanged/worsened lesions** at 3 months [p=0.011 (OR 0.45, 95%CI 0.24-0.84)]; previous anti-TNF exposure, disease duration and therapy optimization during the study were associated with a higher risk of worsened lesions at 3 months [p=0.048 (OR 1.93, 95%CI 1-3.7)] [p=0.006 (OR 2.68, 95%CI 1.32-5.43)] [p=0.011 (OR 3.63, 95%CI 1.34-9.81, respectively)]. At 12 months all these predictors for higher risk of **unchanged/worsened lesions** were confirmed [p=0.04 (OR 2.14, 95%CI 1.02-4.47)] [p=0.006 (OR 3.33, 95%CI 1.40-7.94)] [p=0.02 (OR 3, 95%CI 1.16-7.75, respectively)] (Table 5).

In the multivariable analysis, colonic lesion was associated with a higher risk of **TH** at 3 months [p=0.03 (OR 3.18, 95%CI 1.11-9.10)]; a greater BWT at baseline was the only independent factor associated with a lower risk of **TH** at 3 and 12 months [p=0.035 (OR 0.70, 95%CI 0.5-0.97)] [p=0.011 (OR 0.58, 95%CI 0.38-0.89), respectively] (Table 4). At 3 months therapy optimization during the study was the only independent factor associated with a higher risk of **unchanged/worsened lesions** [p=0.02 (OR 3.34, 95%CI 1.18-9.47)]. At 12 months the only predictor for higher risk of **unchanged/worsened lesions** was disease duration [p=0.02 (OR 3.03, 95%CI 1.15-7.94)] (Table 5).

Discussion

Transmural inflammation is often associated with structural damage that does not completely resolve after treatment[11]. Transmural healing defined as healing of the entire thickness of the intestinal wall of all inflamed segments involved[2,12], has been proposed as a new treatment target in CD[13]. Different studies have demonstrated that TH could be a robust but puzzling endpoint that can be achieved in about a quarter of patients treated with anti-TNF agents [2,14,15]. Furthermore Zorzi and colleagues demonstrated that ultrasonographic response is noted in more than 50% of patients after one year anti-TNF therapy and this response is associated with significantly reduced long-term risk of corticosteroid need, hospitalizations, and/or surgeries among patients with CD[5]. The increasing use of BUS in CD has introduced new challenges, including how to define TH, partial utrasonographic remission and how to interpret discrepancies between endoscopic healing and persistence of transmural abnormalities in BUS assessments. Residual mural abnormalities, such as wall thickening, or extramural lesions can persist in intestinal segments and it is crucial to determine whether these lesions represent established persistent activity that can heal with appropriate biological therapies[16]. Furthermore, understanding the baseline features that may predict the persistence of lesions may be helpful in better understanding post-treatment findings.

A large multicenter German study has been conducted to determine the role of BUS for monitoring treatment response. In this trial, CD patients with acute disease received anti-inflammatory treatment. Almost all sonographic parameters determined during BUS showed a highly significant decrease at different sites[3]. The development of a common US imaging interpretation pattern among sonographers around the world assessing patients with CD may promote this technique as a useful tool in IBD centers for diagnosing suspected CD, determining the extent and severity of mucosal inflammation, evaluating disease activity, monitoring disease course during therapy and postoperative recurrence. Our multicenter study demonstrated that after 3 months from biological therapies initiation TH and improved lesions were reached in 53% of patients, after 6 months in 62.5% of patients and after one year in 64% of patients. After 12 months of therapy the average number of patients who need to be treated to have TH is 3.6 and about 48% of patients with colonic lesions experienced normalization of all ultrasonographic parameters. In the multivariable analysis, colonic lesion was associated with a higher risk of TH at 3 months, as previously observed[17]. The exact reason for the different behavior of the ileum versus parts of the colon on biological treatment evaluated by BUS examination remains unclear. The slower normalization of sonographic parameters, such as BWT at the ileum level compared with colon, is in line with our study[3]. Structural anatomic characteristics, such as higher distribution of Peyer patches or different bacterial colonization at the terminal ileum, could contribute to these differences in sonographic response to treatment. The main ultrasonographic parameter is pre-treatment mural thickness; the greater the thickness before treatment, the higher the risk of incomplete normalization of the wall after treatment. BUS is not only helpful in assessing TH but also can be useful in guiding the choice of optimizing therapy. Although at 3 months therapy optimization during the study was the only independent factor associated with a higher risk of worsened lesions, overall dose escalation induced lesions improvement in 41% of patients. Twenty two percent had TH/improved lesions after 3 months of biological therapy but no ultrasonographic improvement or loss of initial ultrasonographic response at 12 months. On the basis of these results, BUS appears to have an indisputable role in the assessment of lesion remodeling during treatment. No statistical differences in terms of improvement/TH for complications were observed at each time point probably due to a reduced number of patients with these features.

No statistically significant difference in speed of effectiveness of TH among therapies was observed. At 12 months patients treated with ustekinumab showed higher rates of unchanged/worsened lesions than patients treated with anti-TNFs. This observation could be sustained by the patients' number among the four groups of treatments and by the refractory diseases in patients treated with ustekinumab. In our multicentric study, 36% of patients had a prior therapy with anti-TNF agents and treatment with other class of biological drugs could be the final therapeutic option. Patient profiling and personalized therapy to different stages of inflammation will probably allow a better differentiation of ultrasonographic response to biological drugs. However, to our knowledge this is the first study that allows an evaluation of the achievement of transmural healing with different biological drugs.

Some limitations of this study should be acknowledged. Firstly, we assessed transmural healing after different biological therapies on BUS and did not perform a cross-sectional imaging evaluation of transmural healing or endoscopy. Previous studies have demonstrated that using BUS, TH was observed in 25% of patients treated with anti-TNFs, while using MR-Enterography, TH was observed in 23% ($k=0.90$; $p < 0.01$)[15]. Comparison between BUS and endoscopy has already been provided by several previous studies[2,15,18]. The lack of a reference standard to confirm BUS results is not a limitation of this study as our aim was assessing the changes in ultrasonographic parameters after different therapies in patients with established CD where site and extent of the lesions were well-known.

Another limitation may be the potential interobserver and inter-equipment variability that has always been raised as an issue when using US examination. Agreement in scoring of individual parameters especially for BWT is particularly relevant in BUS[6]. However, in our country, the use of BUS is part of standard of care in CD evaluation and is crucial in quickly resolving diagnostic questions and directing physicians to the most appropriate management. In this study BUS was considered part of CD assessment and therapeutic choices and sonographers (gastroenterologists specialized in IBD) contributed to patient management, just as endoscopists performed the procedures with clinical information. All the gastroenterologists were involved in definition and standardization of US parameters reaching a consensus about all lesions and discussing representative examples of the lesions. Further limit was that in Italy TDM is not routinely available and optimization based on clinical management is common.

Although it raises a variety of questions that hopefully will be answered in further multicenter studies, this multicenter study presents data to demonstrate the magnitude and significance of BUS as an effective and easy-to-use tool in tight control and monitoring Crohn's disease lesions during different biological therapies.

Legend tables and figures

Table 1. Characteristics of the study population

Table 2. BUS parameters at baseline and at 3, 6, and 12 months

Table 3. Lesion changes in terms of TH, improved lesions or unchanged/worsened lesions, clinical activity and biomarkers at different time from enrolment

Table 4. Univariable and multivariable analysis showing variables associated with TH at 3 and 12 months

Table 5. Univariable and multivariable analysis showing variables associated with unchanged/worsened lesions at 3 and 12 months

Table 1 supplementary material. Clinical differences among patients in the four groups of treatment

Figure 1. Panel a: transmural healing in terms of BWT, lesion extent, penetrating complications and vascular signals of CD patient of the distal ileum before and after 3 and 12 months of infliximab

Panel b: unchanged lesion in terms of BWT, lumen narrowing, lesion extent and vascular signals of CD patient of the distal ileum before starting treatment and after 3 and 12 months of adalimumab

Figure 2. Percentages of patients achieving TH (panel a), lesion improvement (panel b) or unchanged/worsened lesions (panel c) during the study using different biological therapies; Panel d: rates of TH, improved lesions and unchanged/worsened lesions among the four groups of treatment at 3, 6 and 12 months

Figure 1 supplementary material. Disposition of included and non-included patients

Figure 2 supplementary material. Clinical and biochemical remission among patients achieving TH,

improved lesions or unchanged/worsened lesions

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	n=188
Sex, n (%) *male*	 116 (62%)
Smoking habits, n (%) *yes*	 71 (38%)
CD duration, months *median (range)*	 72 (3- 636)
Age at diagnosis, n (%) - *A1* - *A2* - *A3*	 23 (12%) 132 (70%) 33 (18%)
CD behaviour, n (%) - *B1* - *B2* - *B3*	 80 (43%) 76 (40%) 32 (17%)
CD location, n (%) - *L1* - *L2* - *L3* - L1+L4	 89 (47.4%) 10 (5.3%) 87 (46.3%) 2 (1%)
Perianal disease, n (%) *yes*	 20 (11%)
Prior CD related surgery, n (%) *yes*	 85 (45%)
Prior anti-TNFs, n (%) *yes*	 68 (36%)
Indication to biological therapy at enrolment, n (%) *steroid dependent* *active disease*	 26 (14%) 162 (86%)
Harvey Bradshaw index at enrolment *median (range)*	 7 (0 - 18)
Biological therapy started, n (%) - i*nfliximab* - *adalimumab* - *ustekinumab* - *vedolizumab*	 31 (16%) 103 (55%) 30 (16%) 24 (13%)
Concomitant medication at first dose of biological therapy, n (%) - n*one* - *thiopurines or methotrexate* - *glucocorticoid* - *aminosalicylate drug*	 93 (49.5%) 14 (7.5%) 36 (19%) 45 (24%)
Combo Therapy, n (%)	14 (7%)

BUS Parameters	V0 Baseline n=188	V1 3 mos n=188	V2 6 mos n=171	V3 12 mos n=156	p value V1 vs V0 n=188	p value V2 vs V0 n=171	p value V3 vs V0 n=156
BWT Median, range (mm)							
- Ileal disease	6 (3.4-11.5)	5.5 (3-10)	5 (3-10)	5 (3-9)	p<0.0001	p<0.0001	p<0.0001
- Colonic disease	6.35 (4.3-9)	5.5 (4-8)	4.9 (4-8)	4 (4-8)	p=0.07	p=0.01	p=0.0004
Lesion length Median, range (cm)							
- Ileal disease	15 (4-60)	10 (0-60)	10 (0-60)	10 (0-50)	p=0.009	p=0.0008	p<0.0001
- Colonic disease	40 (20-100)	30 (0-100)	20 (0-100)	10 (0-100)	p=0.98	p=0.0015	p=0.004
Echo pattern							
- Ileal disease					p=0.033	p=0.0046	p=0.0017
• Preserved	105	122	118	110			
• Not preserved	53	36	28	23			
- Colonic disease					p=0.7	p=0.7	p=0.7
• Preserved	23	24	19	19			
• Not preserved	7	6	6	4			
Blood flow Limberg score							
- Ileal disease					p<0.0001	p<0.0001	p<0.0001
• 1	33	69	79	81			
• 2	59	49	42	27			
• 3	39	31	11	18			
• 4	27	9	14	7			
- Colonic disease					p=0.0056	p=0.0017	p=0.015
• 1	8	16	15	14			
• 2	11	8	7	6			
• 3	4	4	3	3			
• 4	7	2	0	0			
Stenosis with dilatation					p=0.6	p=0.8	p=0.2
- Ileal disease	13	11	13	12			
- Colonic disease	0	0	0	1			
Fissures					p=0.3	p=0.5	p=0.012
- Ileal disease	16	11	11	4			
- Colonic disease	1	1	1	0			
Fistula					p>0.99	p=0.2	p=0.3
- Ileal disease	2	2	5	4			
- Colonic disease	0	0	0	0			
Abscess					p>0.99	p=0.3	p>0.99
- Ileal disease	0	0	1	0			
- Colonic disease	0	0	0	0			
Lymph node					p=0.009	p=0.0005	p<0.0001
- Ileal disease	80	58	43	33			
- Colonic disease	11	8	9	5			
Fibrofatty proliferation					p=0.0002	p<0.0001	p<0.0001
- Ileal disease	94	64	57	44			
- Colonic disease	15	9	6	6			

Abbrevations: mos=months; BUS= bowel ultrasonography; BWT=bowel wall thickening

	Baseline	3 mos	6 mos	12 mos	V1 vs V0	V2 vs V0	V3 vs V0
	n=188	n=188	n=171	n=156	n=188	n=171	n=156
Pts with TH							
- Total pts	--	31/188	42/171	43/156	**p<0.0001**	**p<0.0001**	**p<0.0001**
- Ileal disease	--	23/158	32/146	32/133	NNT 6.1	NNT 4.1	NNT 3.6
- Colonic disease	--	8/30	10/25	11/23	(4.6-9.8)	(3.2-5.8)	(2.9-5.1)
Pts with improved lesions							
- Total pts	--	69/188	65/171	56/156	**p<0.0001**	**p<0.0001**	**p<0.0001**
- Ileal disease	--	64/158	60/146	54/133	NNT 2.7	NNT 2.6	NNT 2.9
- Colonic disease	--	5/30	5/25	5/23	(2.3-3.4)	(2.2-3.3)	(2.4-3.8)
Pts with unchanged or worsened lesions							
- Total pts	--	88/188	64/171	54/156	**p<0.0001**	**p<0.0001**	**p<0.0001**
- Ileal disease	--	71/158	54/146	48/133			
- Colonic disease	--	17/30	10/25	6/23			
HBI							
- Remission	16/188	104/188	115/171	116/156	**p<0.0001**	**p<0.0001**	**p<0.0001**
- Active disease	172/188	84/188	56/171	40/156			
Fecal calprotectin							
- Negative	74/170	106/152	108/150	101/130	**p<0.0001**	**p<0.0001**	**p<0.0001**
- Positive	96/170	46/152	42/150	29/130			
C-reactive protein							
- Negative	83/183	121/181	102/159	99/146	**p<0.0001**	**p=0.0005**	**p<0.0001**
- Positive	100/183	60/181	57/159	47/146			

Abbrevations: mos=months; pts=patients; TH=transmural healing; NNT=number need to treat; HBI= Harvey Bradshow Index

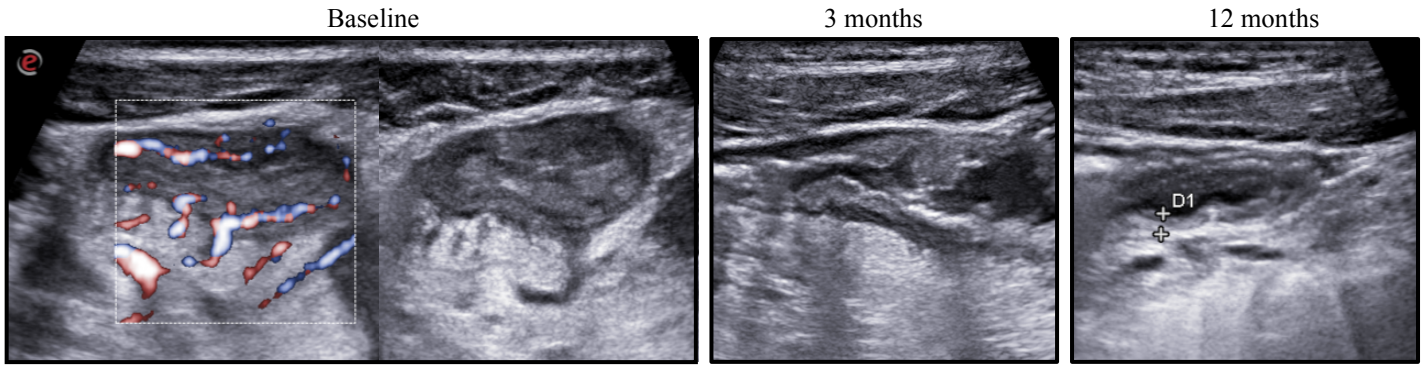
Predictors		Univariable			Multivariable		
		P value	**OR**	**95% CI**	**P value**	**OR**	**95% CI**
TH at 3 mos	**Disease site evaluated at US** (ileal disease ref.) - colonic disease	0.05	2.52	0.97-6.53	**0.027**	**3.34**	**1.15-9.72**
	Prior surgery (no ref.)	0.51	0.76	0.34-1.71	0.5	0.72	0.28-1.85
	Prior TNFs (no ref.)	0.73	0.86	0.36-2.04	0.84	0.88	0.25-3.11
	Disease duration (</= 24 months ref) - >24 mos	0.57	0.78	0.33-1.83	0.57	0.75	0.27-2.07
	Type of biological therapies (IFX ref.)						
	- adalimumab	0.8	1.16	0.35-3.81	0.75	1.24	0.33-4.40
	- vedolizumab	0.52	0.55	0.09-3.37	0.65	0.65	0.10-4.32
	- ustekinumab	0.52	1.58	0.39-6.42	0.48	1.79	0.35-9.09
	BWT (mm)	**0.018**	**0.69**	**0.5-0.94**	**0.047**	**0.71**	**0.51-1**
	Dose escalation during treatment (no ref.)	0.12	0.2	0.03-1.53	0.16	0.23	0.03-1.87
TH at 12 mos	**Disease site evaluated at US** (ileal disease ref.) - colonic disease	**0.02**	**3.14**	**1.14-8.65**	0.67	1.38	0.3-6.28
	Prior surgery (no ref.)	0.28	1.52	0.71-3.27	0.55	0.73	0.25-2.1
	Prior TNFs (no ref.)	0.57	0.79	0.35-1.78	0.85	1.14	0.27-4.82
	Disease duration (</= 24 mos ref) - >24 mos	0.44	0.73	0.33-1.61	0.99	1.01	0.32-3.21
	Type of biological therapies (IFX ref.)						
	- adalimumab	0.05	0.37	0.14-1.02	0.58	0.67	0.17-2.74
	- vedolizumab	0.13	0.37	0.1-1.38	0.41	0.44	0.06-3.22
	- ustekinumab	**0.04**	**0.24**	**0.06-0.97**	0.78	0.77	0.12-5.17
	BWT (mm)	**0.006**	**0.65**	**0.48-0.89**	**0.02**	**0.61**	**0.40-0.93**
	Dose escalation during treatment (no ref.)	0.06	0.24	0.05-1.07	0.27	0.31	0.04-2.59

Abbreviations:
OR=odds ratio; CI= confidence interval; mos= months; IFX=infliximab; BWT=bowel wall thickening; TH=transmural healing.

Predictors		Univariable			Multivariable		
		P value	**OR**	**95% CI**	**P value**	**OR**	**95% CI**
Unchanged/ Worsened lesion at 3 mos	**Disease site evaluated at US** (ileal disease ref.) - colonic disease	**0.006**	**2.68**	**1.32-5.43**	0.28	1.66	0.65-4.23
	Prior surgery (no ref.)	**0.011**	**0.45**	**0.24-0.84**	0.07	0.53	0.26-1.08
	Prior TNFs (no ref.)	**0.048**	**1.93**	**1-3.7**	0.81	1.11	0.46-2.71
	Disease duration (</= 24 mos ref) - >24 mos	**0.006**	**2.68**	**1.32-5.43**	0.10	1.94	0.87-4.29
	Type of biological therapies (IFX ref.)						
	- adalimumab	0.25	1.72	0.67-4.37	0.11	2.31	0.82-6.5
	- vedolizumab	0.16	2.34	0.70-7.7	0.17	2.48	0.67-9.13
	- ustekinumab	**0.03**	**3.4**	**1.07-10.77**	0.14	2.71	0.72-10.20
	BWT (mm)	0.55	0.94	0.77-1.16	0.54	0.93	0.74-1.18
	Dose escalation during treatment (no ref.)	**0.011**	**3.63**	**1.34-9.81**	**0.02**	**3.44**	**1.21-9.82**
Unchanged/ Worsened lesion at 12 mos	**Disease site evaluated at US** (ileal disease ref.) - colonic disease	**0.006**	**3.33**	**1.40-7.94**	0.13	0.37	0.10-1.35
	Prior surgery (no ref.)	0.31	0.70	0.35-1.41	0.53	1.31	0.56-3.05
	Prior TNFs (no ref.)	**0.04**	**2.14**	**1.02-4.47**	0.56	1.37	0.47-3.97
	Disease duration (</= 24 mos ref) - >24 mos	**0.006**	**3.33**	**1.40-7.94**	**0.022**	**3.12**	**1.18-8.3**
	Type of biological therapies (IFX ref.)						
	- adalimumab	0.25	2	0.61-6.59	0.39	1.74	0.48-6.25
	- vedolizumab	0.08	3.48	0.86-14.11	0.10	3.44	0.76-15.56
	- ustekinumab	**0.03**	**4.25**	**1.08-16-77**	0.23	2.61	0.53-12.91
	BWT (mm)	0.6	0.94	0-73-1.20	0.29	0.85	0.63-1.15
	Dose escalation during treatment (no ref.)	**0.02**	**3**	**1.16-7.75**	0.053	2.81	0.99-8.02

Abbreviations:
OR=odds ratio; CI= confidence interval; mos= months; IFX=infliximab; BWT=bowel wall thickening; TH=transmural healing.

A



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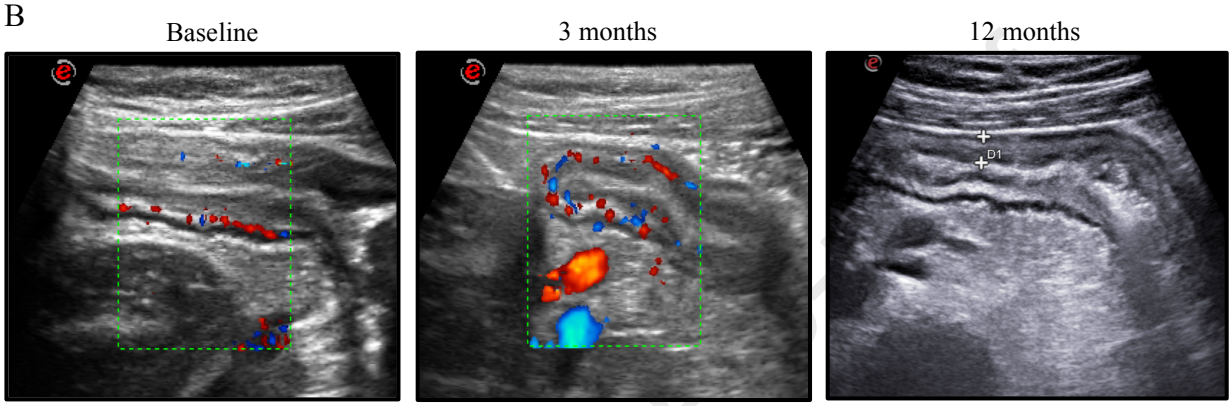
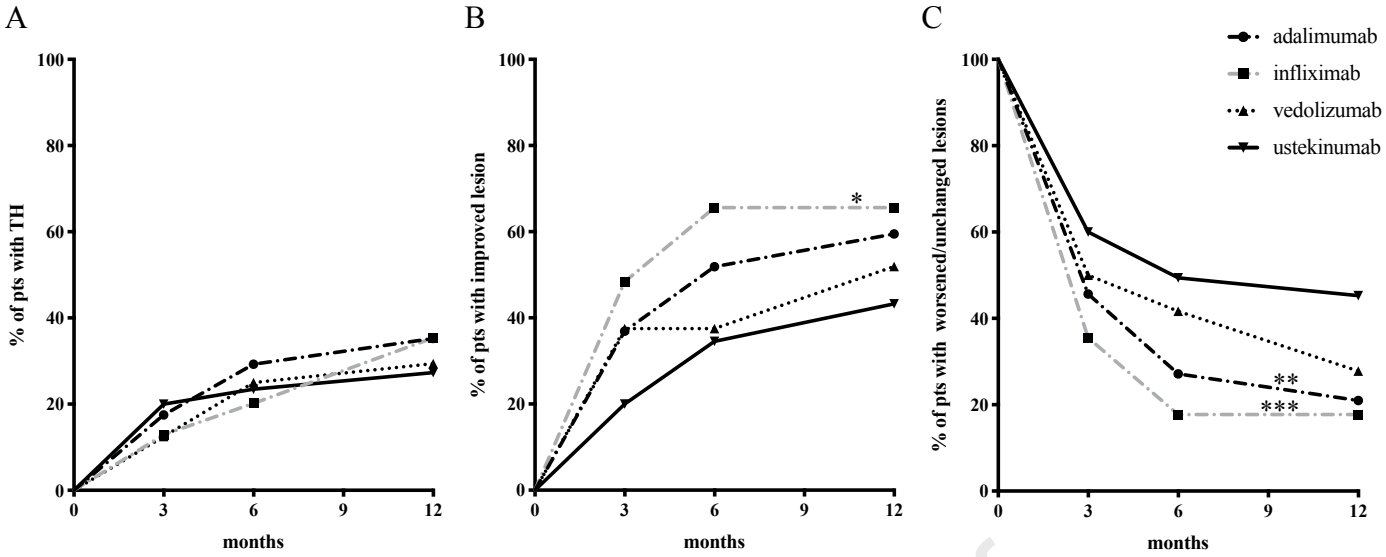
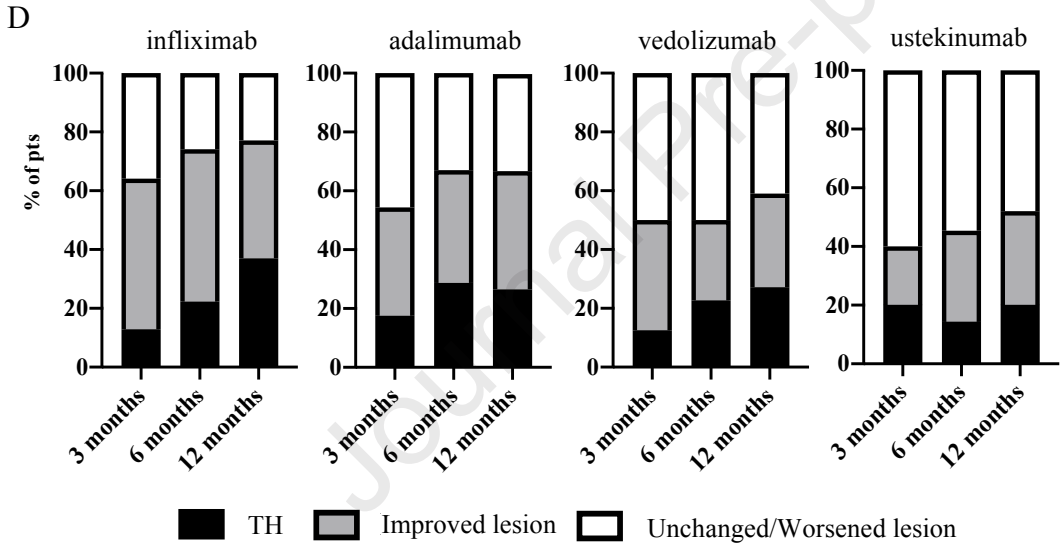




* HR ifx vs ust = 0.4 (95%Cl 0.2-0.9); p=0.037
** HR ada vs ust = 2.1 (95%Cl 1.12-3.9); p=0.02
*** HR ifx vs ust = 2.7 (95%Cl 1.9-6.4); p=0.017



What You Need to Know

Background

The increasing use of bowel ultrasonography in Crohn's disease has introduced new challenges, including how to interpret lesion changes induced by anti-inflammatory therapies (corticosteroids, immunosuppressants and biological drugs) with different ultrasonographic techniques and how to define remission after treatments

Findings

This study demonstrates that, even after few months of treatment with biologics, bowel ultrasonography is useful to individuate transmural healing and significant improvements of the lesions.

Implication for patients care

These results highlight the importance of monitoring biologics-induced bowel lesion improvement/resolution in Crohn's disease using bowel ultrasonography