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Defining the ultrasound longitudinal natural history of newly diagnosed pediatric small bowel Crohn disease treated with infliximab and infliximab–azathioprine combination therapy

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Abstract

Background—Little is known about changes in the imaging appearances of the bowel and mesentery over time in either pediatric or adult patients with newly diagnosed small bowel Crohn disease treated with anti-tumor necrosis factor-alpha (anti-TNF- α) therapy.

Objective—To define how bowel ultrasound findings change over time and correlate with laboratory inflammatory markers in children who have been newly diagnosed with pediatric small bowel Crohn disease and treated with infliximab.

Materials and methods—We included 28 pediatric patients treated with infliximab for newly diagnosed ileal Crohn disease who underwent bowel sonography prior to medical therapy and at approximately 2 weeks, 1 month, 3 months and 6 months after treatment initiation; these patients also had laboratory testing at baseline, 1 month and 6 months. We used linear mixed models to compare mean results between visits and evaluate whether ultrasound measurements changed over time. We used Spearman rank correlation to assess bivariate relationships.

Results—Mean subject age was 15.3 ± 2.2 years; 11 subjects were girls (39%). We observed decreases in mean length of disease involvement (12.0 ± 5.4 vs. 9.1 ± 5.3 cm, $P=0.02$), maximum bowel wall thickness (5.6 ± 1.8 vs. 4.7 ± 1.7 mm, $P=0.02$), bowel wall color Doppler signal (1.7 ± 0.9 vs. 1.2 ± 0.8 , $P=0.002$) and mesenteric color Doppler signal (1.1 ± 0.9 vs. 0.6 ± 0.6 , $P=0.005$) at approximately 2 weeks following the initiation of infliximab compared to baseline. All laboratory inflammatory markers decreased at 1 month (P -values < 0.0001). There was strong correlation

Compliance with ethical standards

Conflicts of interest Dr. Dillman and Dr. Trout received grant funding from Siemens Medical Solutions USA.

between bowel wall color Doppler signal and fecal calprotectin ($P=0.710$; $P<0.0001$). Linear mixed models confirmed that maximum bowel wall thickness ($P=0.04$), length of disease involvement ($P=0.0002$) and bowel wall color Doppler signal ($P<0.0001$) change over time in response to infliximab, when adjusted for age, sex, azathioprine therapy, scanning radiologist and baseline short pediatric Crohn's disease activity index score.

Conclusion—The ultrasound appearance of the bowel changes as early as 2 weeks after the initiation of infliximab therapy. There is strong correlation between bowel wall color Doppler signal and fecal calprotectin.

Keywords

Anti-tumor necrosis factor-alpha; Biological therapy; Children; Crohn disease; Infliximab; Small bowel; Ultrasound

Introduction

An estimated 25–30% of individuals with Crohn disease are diagnosed during childhood or adolescence, with involvement most commonly affecting the distal small bowel and colon [1–3]. Timely diagnosis and treatment of this condition is critical because misdiagnosis or under-treatment has been associated with significant co-morbidities, including impaired growth and failure to thrive, development of disease-related complications (e.g., intra-abdominal abscess and bowel obstruction), and overall reduced health-related quality of life [4–7].

Over the last decade, the introduction of anti-tumor necrosis factor-alpha (anti-TNF- α) medications, or so-called biological therapies, has substantially altered the management of Crohn disease in the pediatric population. Pediatric gastroenterologists are increasingly embracing these medications (e.g., infliximab) as a first-line therapy in children with evidence of moderate and severe disease activity [8]. When compared to other monotherapies, such as immunomodulators (e.g., azathioprine), anti-TNF- α medications have been shown to have similar or better efficacy (including length of long-term remissions) as well as an acceptable safety profile, and might result in decreased need for corticosteroids and radiologic studies (with resultant decreased ionizing radiation exposure) [9–16].

While CT and MRI are the most often used imaging modalities for assessing the bowel and mesentery of Crohn disease in the United States, ultrasound also can be employed [17–25]. Ultrasound (US) has several advantages compared to CT, including lower cost, lack of ionizing radiation, and potentially no need for oral or intravascular contrast materials. Ultrasound's advantages compared to MRI include lower cost, shorter examination length, and potentially no need for oral or intravascular contrast materials. Anti-peristaltic agents are also commonly used for MRI of the bowel and are not needed when imaging with ultrasound [26]. Because of these advantages, ultrasonography can be performed more frequently in children with Crohn disease in both the clinical and research settings to assess changes in the appearances of the bowel and mesentery over time and in response to medical therapy.

This is important because little is known about changes in the imaging appearances of the bowel and mesentery over time in either children or adults with newly diagnosed small bowel Crohn disease treated with anti-TNF- α therapy. Therefore, the primary objective of our study was to define, using ultrasound, the longitudinal natural history of the appearance of the small bowel and mesentery in children with newly diagnosed pediatric small bowel Crohn disease managed with infliximab over the first 6 months of treatment. Such an investigation is needed to show that ultrasound (and imaging, in general) can serve as a radiologic biomarker for establishing response to therapy. We also sought to determine the strength of correlations between imaging findings and laboratory markers of inflammation and a validated Crohn disease activity survey instrument. Finally, as an exploratory aim, we wanted to determine whether changes in the appearance of the bowel over time differ based on patient age, gender, or the addition of azathioprine therapy (combination therapy).

Materials and methods

Our institutional review board approved this prospective observational study, which complies with the Health Insurance Portability and Accountability Act and is registered at www.clinicaltrials.gov. We obtained parent or guardian informed consent and subject assent for all participants. All subjects in the current study were previously reported in publications evaluating (1) ultrasound and MRI inter-radiologist agreement in pediatric small bowel Crohn disease [25] and (2) change in MRI bowel wall diffusion-weighted imaging apparent diffusion coefficient (ADC) values over time in response to infliximab therapy [27], whereas in this manuscript we attempt to define, using ultrasound, the longitudinal natural history of the appearance of the small bowel and mesentery in children who were newly diagnosed with small bowel Crohn disease managed with infliximab over the first 6 months of treatment.

Between July 2012 and December 2014 at the University of Michigan C. S. Mott Children's Hospital, we prospectively enrolled 28 pediatric patients (age eligibility range, 8–18 years) treated with infliximab (Remicade; Janssen Biotech, Horsham, PA) for newly diagnosed biopsy-proven ileal Crohn disease. A total of 46 potential subjects were approached; 18 declined to participate. Exclusion criteria included:

- prior diagnosis of inflammatory bowel disease,
- any medical or surgical treatment for Crohn disease prior to baseline study imaging,
- Crohn disease without involvement of the distal small bowel or
- inability to obtain parent/guardian informed consent or subject informed assent.

The decisions to treat subjects with infliximab were strictly clinical and made by the subjects' pediatric gastroenterologists. Infliximab induction and maintenance infusions were performed using standard institutional dosing and timing (5 mg/kg at 0 weeks, 2 weeks and 6 weeks, and every 8 weeks thereafter). Some subjects received combination therapy with concomitant oral azathioprine (Imuran; Prometheus Laboratories, San Diego, CA) at the discretion of the treating pediatric gastroenterologist. The decision to treat some subjects

with combination therapy was based on multiple factors, including severity of disease and physician preference as well as the idea that concomitant immunomodulator therapy might affect a child's ability to remain on long-term biological therapy [28].

Subjects underwent baseline gray-scale and color Doppler US of the bowel wall and mesentery after diagnosis and prior to the first infliximab infusion (baseline imaging, or visit 1) as well as at approximately 2 weeks (± 1 week; visit 2), 1 month (± 2 weeks; visit 3), 3 months (± 2 weeks; visit 4) and 6 months (± 1 month; visit 5) after initiation of infliximab therapy. Laboratory inflammatory testing (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR] and fecal calprotectin) was performed at baseline (visit 1), approximately 1 month (visit 3), and approximately 6 months (visit 5). A previously validated Crohn disease activity survey instrument (based primarily on patient-reported outcomes), the short pediatric Crohn's disease activity index (sPCDAI), also was administered to subjects by a clinical research coordinator at baseline (visit 1), approximately 1 month (visit 3), and approximately 6 months (visit 5). This survey tool asks patients about their general well-being, abdominal pain, stools, weight and extra-intestinal disease manifestations [29].

At each time point, sonography of the bowel (ileum) and its associated mesentery was performed by at least one of three board-certified, fellowship-trained pediatric radiologists (M.A.D., 34 years' post-fellowship training; E.A.S., 5 years' post-fellowship training; R.S., 17 years' post-fellowship training). A second radiologist (one of the three) also scanned, if available, blinded to the results obtained by the first scanning radiologist. The radiologist(s) chosen to scan a given subject for a given encounter was determined by the clinical research coordinator using a non-random process based on physician availability. Radiologists were not told whether imaging was for baseline evaluation or for follow-up after treatment initiation; however, it is possible that radiologists increasingly recognized subjects (and their bowel) over time because they were unable to be completely blinded. The distal/terminal ileum was identified using a variety of anatomical landmarks, such as the ascending colon, ileocecal valve and vermiform appendix.

Study radiologists documented the following for each US examination:

- length of involvement of the abnormal distal/terminal ileal Crohn disease segment, obtained using summated linear measurements from one or more still gray-scale images (longest contiguous segment, rounded to the nearest cm);
- maximum bowel wall thickness of the diseased distal/terminal ileal segment, measured from mucosal to serosal surface (in mm);
- amount of bowel wall color Doppler signal, on average (0–3 Likert-like scale, where 0 = no detectable color Doppler signal in the bowel wall and 3 = markedly increased blood flow with contiguous/near-contiguous circumferential bowel wall Doppler signal); and
- amount of mesenteric color Doppler signal adjacent to the abnormal ileal bowel segment, on average (0–3 Likert-like scale, where 0 = no detectable color Doppler signal and 3 = markedly increased blood).

All US imaging was performed using the same Acuson S3000 ultrasound system (Siemens Medical Solutions USA, Malvern, PA). Study radiologists had access to multiple US transducers, including linear high-frequency (e.g., 9L4) and curved low-frequency (e.g., 6C2) transducers. Gray-scale imaging was performed using graded compression technique over the abdomen in the location of the distal/terminal ileum, typically the right lower quadrant. No intravenous or oral contrast materials were administered. Color Doppler imaging was performed using a 9L4 transducer while selecting the lowest pulse repetition frequency that maximized signal while preventing aliasing. Color Doppler gain was set by turning up the gain until random noise was noted on the images and then lowering the gain until the noise disappeared. In instances when there was more than one abnormal segment of distal ileum, the longest segment of contiguous abnormality was selected for study US imaging.

Demographic data were recorded, including sex and age at the time of study enrollment.

For encounters where two radiologists scanned a subject, the results for each US measurement were averaged to come up with a single measurement for the visit. Continuous data were summarized as means and standard deviations, while categorical data were summarized as counts and percentages. Spearman rank correlation (ρ) was used to assess bivariate relationships (e.g., US measurements vs. laboratory values). Continuous results were compared between visits using linear mixed models.

Linear mixed models also were used to evaluate the effect of time (in days) on US measurements as a response to infliximab therapy, adjusted for numerous covariates, including subject age, sex, azathioprine therapy, baseline sPCDAI and scanning radiologist [30]. For these analyses, we were able to use both radiologists' measurements (instead of the average measurement) when two radiologists scanned a given encounter. All models used an unstructured covariance matrix and included a random intercept term. Final models were selected based on Akaike Information Criterion (AIC), which is used to evaluate model goodness of fit [30]. Mixed models were employed to take into account the correlation within each subject. These models also can tolerate unbalanced data (differences in the exact day of follow-up between subjects as well as data "missing at random") and use all available measurements in our analyses [31].

A P -value <0.05 was considered significant for inference testing. Correlation coefficients were classified using the following definitions: 0–0.19 = very weak, 0.2–0.39 = weak, 0.40–0.59 = moderate, 0.60–0.79 = strong, and 0.80–1.0 = very strong [32]. A priori sample size calculation for bivariate correlation analyses assuming a null correlation of 0, expected correlation coefficient of 0.5, $\alpha = 0.05$ (two-tailed), and 85% power was $n=33$. No formal a priori power analysis was performed for linear mixed-model analyses because these analyses were considered exploratory.

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Results

Over the study period, at total of 119 US encounters occurred in 28 subjects (4.25 encounters per subject, on average). Mean subject age was 15.3 ± 2.2 years (range, 9–18 years) at the time of enrollment, and 11 subjects were girls (39%). Fifteen of 28 (54%) subjects received azathioprine. Visits 2, 3, 4, and 5 actually occurred at means of 16.2 ± 3.5 days, 39.3 ± 8.8 days, 102.3 ± 8.4 days and 193.1 ± 16.4 days after the initiation of infliximab therapy.

Eight subjects were missing at least some follow-up US data because they were either lost to follow-up or non-compliant with the study protocol ($n=4$) or they underwent surgical resection of the small-bowel segment of interest ($n=4$). Three subjects who underwent surgery completed visits 1, 2, and 3, while a single subject had only visit 1 baseline data. Some subjects were missing small amounts of laboratory or sPCDAI data for the same reasons mentioned above. Specifically, 21/140 (15.0%) planned US encounters were missing for the reasons mentioned above (e.g., surgical bowel resection). Subjects were scanned by two radiologists at 101/119 (84.9%) US visits.

Ultrasound results by visit are provided in Table 1. The change in imaging findings over time in response to infliximab therapy is presented in box plots (Fig. 1). When compared to baseline and using linear mixed models, significant decreases in US mean length of disease involvement (12.0 ± 5.4 vs. 9.1 ± 5.3 cm, $P=0.02$), maximum bowel wall thickness (5.6 ± 1.8 vs. 4.7 ± 1.7 mm, $P=0.02$), bowel wall color Doppler signal (0–3, where 0 = no detectable color Doppler signal and 3 = markedly increased blood flow; 1.7 ± 0.9 vs. 1.2 ± 0.8 , $P=0.002$) and mesenteric color Doppler signal (0–3, where 0 = no detectable color Doppler signal and 3 = markedly increased blood flow; 1.1 ± 0.9 vs. 0.6 ± 0.6 , $P=0.005$) were noted at approximately 2 weeks (visit 2) following the initiation of infliximab therapy compared to baseline (Fig. 2). These significant decreases relative to baseline persisted throughout follow-up.

Laboratory and sPCDAI results by visit are provided in Table 2. Laboratory values and sPCDAI over time in response to therapy are presented in box plots (Fig. 3). When compared to baseline and using linear mixed models, all three laboratory inflammatory markers as well as the sPCDAI showed highly significant decreases at visit 3, or approximately 1 month after the initiation of therapy (P -values < 0.0001). These significant decreases persisted (again compared to baseline) at visit 5, or approximately 6 months after the start of treatment.

Bivariate correlations

Table 3 shows correlations among US measurements, laboratory results and sPCDAI scores for all visits combined. The greatest correlation between a US finding and a laboratory inflammatory marker was between bowel wall color Doppler signal and fecal calprotectin ($\rho=0.710$; $P<0.0001$; Fig. 4).

Linear mixed models to assess change over time

Linear mixed models confirmed that maximum bowel wall thickness ($P=0.04$), length of disease involvement ($P=0.0002$) and bowel wall color Doppler signal ($P<0.0001$) changed

over time in response to infliximab therapy, adjusted for subject age, sex, azathioprine therapy, scanning radiologist and baseline sPCDAI score. For example, bowel wall thickness decreased 0.004 mm per day in response to infliximab therapy, on average, after adjustment for other covariates. Time was not a significant covariate when evaluating change in mesenteric color Doppler signal over time after covariate adjustment ($P=0.09$). Azathioprine combination therapy did not significantly affect any US measurement assessed (P -values >0.05), when controlling for other variables. Table 4 shows parameter estimates, standard errors and P -values for each fixed effect. Interaction terms (covariate*time) were used to test for differences in change in US measurements over time related to age, gender and azathioprine. No interaction term improved the goodness of fit of any model based on AIC.

The reader covariate was significant for three of the four models, indicating that there were significant differences in measurements among readers, on average (Table 4). However, by including the radiologists as a covariate in the model, we were able to adjust our results for reader variability and improve the accuracy of our inferences (i.e. P -values).

Discussion

Our study helps establish the longitudinal natural history of US findings of newly diagnosed small bowel Crohn disease in a pediatric population treated with biological therapy. Our results show that significant changes in the US appearances of the bowel and mesentery occur over time during the first 6 months of treatment with infliximab. On average, it took only 2 weeks to see significant decreases in length of disease involvement, maximum bowel wall thickness, bowel wall color Doppler signal and mesenteric color Doppler signal. Interestingly, the decrease in bowel wall color Doppler signal at 2 weeks was particularly highly significant, and this degree of statistical significance persisted at each follow-up visit. Increased color Doppler bowel wall vessel density has been shown to be associated with increased Crohn disease activity [21]. A study by Paredes et al. [33] in adults on baseline US imaging and additional imaging 2 weeks following induction of anti-TNF- α therapy also showed statistically significant decreases in bowel wall thickening and color Doppler blood flow in response to medical treatment, although imaging at multiple follow-up time points was not performed.

A study by Moreno et al. [34], who performed bowel ultrasound at a minimum of 1 year after the initiation of anti-TNF- α or immunomodulator therapy, showed that US findings could be used to demonstrate evidence of mucosal healing, an increasingly recognized important therapeutic end point. Moy et al. [35] also showed in a retrospective study that follow-up MRI examinations can be used to assess mucosal healing in young patients with Crohn disease. Because subjects in our study did not undergo systematic follow-up endoscopic assessments, further studies with such assessments are needed with to determine whether mucosal healing can be demonstrated noninvasively by ultrasound earlier than 1 year, perhaps as early as a few weeks to 6 months. A study by Ordás et al. [36] using MRI showed that healing of mucosal ulcers and endoscopic remission could be demonstrated as early as 12 weeks into treatment using corticosteroids or biological therapy.

Our observations might have important implications with regard to the timing of follow-up imaging after the initiation of infliximab in both the clinical and research settings. It is conceivable that follow-up imaging as early as 2 weeks to 1 month could accurately predict ultimate response versus non-response to medical treatment. Such radiologic non-responders might benefit from an early change in their medical management (e.g., change in medication(s), escalation of dose, or initiation of combination therapy), although further studies are needed to specifically investigate this supposition. Our linear mixed models indicate that the changes in US findings over time are not significantly affected by subject age, sex or the addition of azathioprine therapy.

Our results show that laboratory inflammatory markers tend to return to normal or nearly normal by 1 month after the initiation of biologic therapy in the majority of pediatric patients with Crohn disease. While the various US measurements we assessed all seemed to decrease as early as 2 weeks after starting treatment, on average, the degree of “normalization” was less striking (compared to laboratory assessments), with numerous subjects remaining abnormal in appearance over the entire 6-month follow-up period. This apparent ultrasound–laboratory inflammatory marker discordance could be simply because US changes lag behind laboratory changes. Alternatively, it is conceivable that infliximab neutralizes systemic TNF- α and decreases overall inflammation in the body, and thus laboratory inflammatory markers, while the bowel and mesentery remain locally inflamed. Again, further studies with a longer longitudinal horizon would be useful for further characterizing the relationships between imaging findings and laboratory markers of inflammation and how they change over the long run. It is also worth noting that a few subjects had normal laboratory inflammatory results and sPCDAI scores at baseline, despite very abnormal appearance of the bowel at ultrasound. This observation confirms that laboratory testing alone cannot be used to reliably exclude the presence of small bowel Crohn disease.

C-reactive protein and fecal calprotectin showed either moderate or strong correlations with all four US findings evaluated. In particular, fecal calprotectin strongly correlated with length of disease involvement, maximum bowel wall thickness and amount of bowel wall color Doppler signal, with bowel wall color Doppler signal having the highest correlation ($\rho=0.71$). It is conceivable that a simple focused US examination assessing one or two findings (e.g., amount of bowel wall color Doppler signal and maximum bowel wall thickness) and taking just a few minutes could serve as an alternative to fecal calprotectin testing. This might be justifiable based on the fact that fecal calprotectin, a commonly used laboratory marker of intestinal mucosal inflammation [37], involves obtaining a stool sample after the patient has left clinic and transporting it to a laboratory. These realities as well as the fact that patients and parents might be generally uncomfortable with stool testing seem to result in a relatively poorly tolerated test. This was confirmed in our study, where there was much lower subject compliance with fecal calprotectin testing compared to compliance with US imaging or testing of blood inflammatory markers. Twenty-nine percent of subjects in our study with documented same-day CRP and ESR results had missing fecal calprotectin results because of subject non-compliance. Correlations between US findings and erythrocyte sedimentation rate as well as between US findings and sPCDAI scores were only weak to moderate, at best.

Our study has limitations. First, it is relatively small, with only 28 subjects. This being said, it was conducted in a prospective manner with subjects on average obtaining 4.25 of five scheduled follow-up US examinations. A second potential limitation is that study US imaging was performed by a single radiologist at some baseline or follow-up encounters versus by two radiologists at others, based on radiologist availability. We were able to adjust for this concern by treating the scanning radiologists as covariates when performing linear mixed-model analyses. Because we observed very highly significant results with this adjustment, we believe that our study conclusions are sound and accurately reflect (1) how the bowel changes over time in response to infliximab therapy and (2) how US findings correlate with laboratory inflammatory markers. Also, by including the radiologists in the model, we were able to specifically adjust for measurement variation among radiologists, which has been shown to be relatively high for certain US measurements in Crohn disease (e.g., length of disease) [25]. However it is worth noting that complicated regression models such as those used in this study could have limited reliability and validity when sample sizes are relatively small and the number of predictor variables are relatively large.

A third limitation is that eight subjects were missing US, laboratory and sPCDAI follow-up data from one or more visits because they were (1) lost to follow-up or withdrew from our study ($n=4$), or (2) required surgery to remove the small-bowel loop of interest ($n=4$). A few additional subjects were missing small amounts of laboratory or sPCDAI data from a single time point, but these data appeared to be missing at random and were unlikely to systematically impact our results. A fourth limitation is that our first post-infliximab laboratory assessments were obtained at visit 3, or about a month after the start of therapy. We do not have laboratory data to correlate with US findings obtained at 2 weeks (visit 2).

A fifth limitation is that bowel wall and mesenteric color Doppler signal were assessed using semi-quantitative Likert-like scales. Although such scales are commonly employed and have been shown to be useful in other conditions, such as for the prediction of clinically significant prostate cancer at multi-parametric MRI [38], exact definitions for the amount of Doppler signal (e.g., based on vessel density) need to be established for each ordinal value in order to aid generalizability. Such semi-quantitative assessments also should be validated (e.g., Delphi methodology), such as has been recently carried out for the US assessment of synovitis in children [39], and we should be working toward developing more objective outcomes. A final limitation is that we only assessed a single anti-TNF- α medication (infliximab), while other such medications — e.g., adalimumab (Humira; AbbVie, North Chicago, IL) — are available for use in the United States.

Conclusion

Our study helps establish how the US appearances of the bowel and mesentery change over time during the first 6 months of infliximab therapy, including in the setting of infliximab–azathioprine combination therapy. Our results show that ultrasound is a responsive biomarker for assessment of disease activity, with changes observed as early as 2 weeks after the start of treatment, on average. Bowel wall color Doppler signal seems to decrease particularly early after the initiation of infliximab infusions and might be useful as a surrogate for fecal calprotectin testing and as a timely predictor of response versus non-

response, although further investigations are needed to study these possibilities. Further research also is needed to determine whether individual lack of response at ultrasound by approximately 1–3 months should necessitate change in medical therapy, and if changes in the US appearance of the bowel correlate with or predict endoscopic mucosal healing.

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Fig. 1.

Tukey box plots show change in ultrasound findings over time. **a** Length of distal ileal involvement. **b** Maximum bowel wall thickness. **c** Bowel wall color Doppler signal. **d** Mesenteric color Doppler signal. Visit 1 = baseline; visit 2 = approximately 2 weeks; visit 3

= approximately 1 month; visit 4 = approximately 3 months; visit 5 = approximately 6 months. Boxes represent interquartile ranges, while horizontal lines within the boxes represent medians (some medians overlap the x-axis). Circles, triangles and diamonds represent statistical outliers. Boxes in figure parts (c) and (d) that appear to lack a horizontal line (visits 4 and 5) have a median value of zero

Fig. 2.

Baseline and follow-up US imaging in a 16-year-old boy with newly diagnosed distal ileal Crohn disease. **a** Baseline color Doppler image through the right lower quadrant prior to infliximab therapy shows markedly increased blood flow in the bowel wall (scored 3 by both radiologists). **b** Follow-up image from visit 2 shows substantially decreased color Doppler signal in the same loop of bowel following infliximab therapy (scored 1 by both radiologists). Note that imaging planes are slightly different on the images captured

Fig. 3.

Tukey box plots show change in laboratory inflammatory markers and short pediatric Crohn's disease activity index (sPCDAI) over time. **a** C-reactive protein. **b** Erythrocyte sedimentation rate. **c** Fecal calprotectin. **d** sPCDAI. Visit 1 = baseline; visit 3 = approximately 1 month; visit 5 = approximately 6 months. Boxes represent interquartile ranges, while horizontal lines within the boxes represent medians. Circles, squares and triangles represent statistical outliers

Table 1

Ultrasound results by study visit^a

	Visit 1 [n]	Visit 2 ^b (P-value) [n]	Visit 3 ^b (P-value) [n]	Visit 4 ^b (P-value) [n]	Visit 5 ^b (P-value) [n]	Overall ^b P-value
Length of disease involvement (cm)	12.0±5.4 [28]	9.1±5.3 (0.02) [26]	8.0±6.5 (0.001) [24]	4.4±4.4 (<0.0001) [21]	5.4±6.0 (0.0009) [21]	<0.0001
Maximum bowel wall thickness (mm)	5.6±1.8 [28]	4.7±1.7 (0.02) [26]	4.3±2.2 (0.0004) [24]	3.7±2.1 (0.0001) [21]	3.7±2.0 (<0.0001) [21]	<0.0001
Bowel wall color Doppler signal (0–3)	1.7±0.9 [28]	1.2±0.8 (0.002) [26]	1.0±1.0 (<0.0001) [24]	0.7±0.9 (<0.0001) [21]	0.7±0.9 (<0.0001) [21]	<0.0001
Mesenteric color Doppler signal (0–3)	1.1±0.9 [28]	0.6±0.6 (0.005) [26]	0.7±0.8 (0.02) [24]	0.4±0.6 (0.001) [21]	0.4±0.6 (0.002) [21]	0.004

^aMean ± standard deviation. Visit 1 = baseline; visit 2 = approximately 2 weeks; visit 3 = approximately 1 month; visit 4 = approximately 3 months; visit 5 = approximately 6 months

^bFollow-up encounter measurements were compared to baseline measurements using linear mixed models. Overall P-values are provided for each imaging finding in addition to individual P-values for each follow-up visit compared to baseline (visit 1). A P-value <0.05 was considered significant for inference testing

Table 2Laboratory and short pediatric Crohn's disease activity index (sPCDAI) results by study visit^a

	Visit 1 [n]	Visit 3 (<i>P</i> -value) [n]	Visit 5 (<i>P</i> -value) [n]	Overall <i>P</i> -value
C-reactive protein (mg/L)	2.9±3.5 [28]	0.4±0.6 (<0.0001) [24]	0.3±0.5 (<0.0001) [21]	<0.0001
Erythrocyte sedimentation rate (mm/h)	27.9±18.6 [27]	10.7±10.8 (<0.0001) [22]	10.7±10.7 (<0.0001) [21]	<0.0001
Fecal calprotectin (µg/g)	672.8±154.4 [19]	358.1±80.1 (<0.0001) [20]	358.4±103.5 (<0.0001) [12]	<0.0001
sPCDAI	35.3±17.0 [28]	13.5±15.5 (<0.0001) [24]	16.4±15.8 (<0.0001) [21]	<0.0001

^aMean ± standard deviation. Visit 1 = baseline; visit 3 = approximately 1 month; visit 5 = approximately 6 months^bFollow-up encounter measurements were compared to baseline measurements using linear mixed models. Overall *P*-values are provided in addition to individual *P*-values for each follow-up visit compared to baseline (visit 1). A *P*-value <0.05 was considered significant for inference testing

Table 3

Correlations (ρ) among ultrasound findings, inflammatory laboratory results and short pediatric Crohn's disease activity index (sPCDAI) results for all visits combined^a

	C-reactive protein (<i>P</i> -value ^b) [<i>n</i>]	Erythrocyte sedimentation rate (<i>P</i> -value ^b) [<i>n</i>]	Fecal calprotectin (<i>P</i> -value ^b) [<i>n</i>]	sPCDAI (<i>P</i> -value ^b) [<i>n</i>]
Length of disease involvement (cm)	0.48 (<0.0001) [72]	0.44 (0.0002) [69]	0.61 (<0.0001) [51]	0.23 (0.049) [72]
Maximum bowel wall thickness (mm)	0.51 (<0.0001) [72]	0.37 (0.002) [69]	0.63 (<0.0001) [51]	0.32 (0.006) [72]
Bowel wall color Doppler signal	0.51 (<0.0001) [72]	0.51 (<0.0001) [69]	0.71 (<0.0001) [51]	0.20 (0.093) [72]
Mesenteric color Doppler signal	0.43 (0.0002) [72]	0.36 (0.002) [69]	0.54 (<0.0001) [51]	0.29 (0.014) [72]

^a Spearman rank correlation coefficients

^b A *P*-value <0.05 was considered significant for inference testing

Table 4

Linear mixed-model fixed-effect parameter estimates for assessing change in ultrasound (US) appearances of the bowel and mesentery over time

US finding modeled	Covariate	Parameter estimate	Standard error	P-value
Length of disease involvement (cm)				
	Intercept	9.98	6.72	0.15
	Time (days from baseline imaging)	−0.03	0.01	0.0002 *
	Age (years)	0.18	0.43	0.67
	Sex (male=1)	−1.15	1.82	0.53
	Imuran	0.78	1.83	0.67
	sPCDAI	0.07	0.03	0.049
	Reader 1	−5.29	1.10	<.0001
	Reader 2	−5.92	1.43	<.0001
	Reader 3	0	.	.
Maximum bowel wall thickness (mm)				
	Intercept	2.99	2.38	0.22
	Time (days from baseline imaging)	−0.004	0.002	0.04 *
	Age (years)	0.04	0.15	0.81
	Sex (male=1)	−0.65	0.60	0.28
	Imuran	0.90	0.66	0.18
	sPCDAI	0.03	0.01	0.002
	Reader 1	0.50	0.30	0.09
	Reader 2	1.99	0.39	<.0001
	Reader 3	0	.	.
Bowel wall color Doppler signal				
	Intercept	2.15	1.26	0.0997
	Time (days from baseline imaging)	−0.003	0.001	<.0001 *
	Age (years)	−0.03	0.08	0.70
	Sex (Male=1)	−0.42	0.30	0.16
	Imuran	0.12	0.35	0.74
	sPCDAI	0.006	0.004	0.19
	Reader 1	−0.32	0.13	0.017
	Reader 2	0.13	0.17	0.45
	Reader 3	0	.	.
Mesenteric color Doppler signal				
	Intercept	1.54	0.96	0.12
	Time (days from baseline imaging)	−0.001	0.001	0.09
	Age (years)	−0.05	0.06	0.46
	Sex (male=1)	−0.52	0.25	0.04

US finding modeled	Covariate	Parameter estimate	Standard error	P-value
	Imuran	0.21	0.26	0.43
	sPCDAI	0.01	0.004	0.01
	Reader 1	0.06	0.14	0.66
	Reader 2	0.01	0.18	0.95
	Reader 3	0	.	.

* US finding significantly decreased over time (a *P*-value <0.05 was considered significant for inference testing). For example, bowel wall thickness decreased 0.004 mm per day (or 0.028 mm per week) in response to infliximab therapy, on average, after adjustment for other covariates *sPCDAI* short pediatric Crohn's disease activity index