Strain elastography in Crohn's disease: the role of visual observation and semiquantitative parameters

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Received: 31 May 2018 / Accepted: 28 September 2018 / Published online: 20 October 2018
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Abstract

Strain elastography is a recently developed ultrasound method that seems to contribute to the diagnosis and monitoring of inflammatory bowel diseases. This pictorial essay aims to present the feasibility of strain elastography in clinical practice by comparing visual observation and semiquantitative parameters with endoscopic or radiological images in some common cases of Crohn's disease. In our opinion, strain elastography with visual observation is easily performed in connection with B-mode ultrasound and color Doppler imaging and may provide relevant information. Strain elastography with the elaboration of semiquantitative parameters is not as immediate as the visual observation method, but it seems to provide more objective data and seems more appropriate for monitoring the evolution of the disease. However, for the elastographic method to extensively contribute in inflammatory bowel diseases, standardization and further studies are required to confirm the promising results already reported in the literature.

Keywords Gastrointestinal ultrasound · Strain elastography · Bowel strain elastography · Crohn's disease

SOMMARIO

L'elastografia Strain è una recente metodica ecografica che sembra avere un ruolo nella diagnosi e nel monitoraggio delle malattie infiammatorie intestinali. Lo scopo di questo Pictorial Essay è di verificare la fattibilità dell'elastografia Strain nella pratica clinica confrontando l'osservazione visiva e i parametri semi-quantitativi con immagini endoscopiche o radiologiche in alcuni casi comuni di malattia di Crohn. A nostro parere, l'elastografia strain con osservazione visiva è facilmente eseguibile in connessione con l'ecografia B-mode ed il color Doppler e può fornire informazioni rilevanti. L'elastografia strain con l'elaborazione di parametri semiquantitativi non è immediata come il metodo di osservazione visiva, ma sembra fornire dati più oggettivi e sembra più appropriata per monitorare l'evoluzione della malattia. Tuttavia, affinché il metodo elastografico possa avere un ruolo più diffuso nelle malattie infiammatorie dell'intestino, sono necessari la standardizzazione del metodo e ulteriori studi che confermino i risultati promettenti già riportati in letteratura.

Introduction

Abdominal ultrasound (US) examination, including investigation of the bowel loops, is commonly used in the diagnosis and monitoring of intestinal inflammatory diseases [1]. In clinical practice, the combination of different US

methods—such as B-mode US, color Doppler (CD), contrast-enhanced US (CEUS), and strain elastography (SE)—provides additional information on the structure of the affected bowel wall (thickness, stratification, macro- and microvascularization, elasticity/stiffness).

SE is a recently developed US method that has proved useful in investigating abdominal pathologies, and detailed information on the physical principles, methods, and clinical applications is available in previous papers [2, 3].

In this paper, we report our experience with SE visual observation in Crohn's disease using Fufezan's classification, which seems useful in some common patterns of this disease [4].

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This pictorial essay aims to present semiquantitative elastography data computation in Crohn's disease and compare the results with those of visual observation and corresponding endoscopic or radiographic pictures.

We reviewed clips obtained in a recent series of patients affected by Crohn's disease and elaborated the most representative images using the scanner software available in our institution (Elaxto, Esaote SpA, Italy) to obtain semiquantitative data (mean, HRD, and elastic ratio values).

The elastography patterns obtained in the initial phase of Crohn's disease are described separately from those obtained in patients with a long-term disease. Crohn's disease can affect any part of the digestive tract, but only the elastography images obtained on the most frequent localizations—that is, the terminal ileum and the proximal colon—are described. In this paper, the elastic ratio is reported only in the sections dealing with bowel stenosis and therapy monitoring.

Examination technique

In our institute, a high-end scanner (Twice, Esaote Biomedica, Italy) and a high-frequency linear probe (LA332, 3–11 MHz) are generally used, but in the study of bowels located deep in the abdomen, a convex probe (CA541, 1–8 MHz) is preferred. Elastography is performed using a standard color-scale map with 100 shades of colors ranging from blue to red (blue for maximum stiffness, green for intermediate stiffness, and red for maximum elasticity).

A good-quality elastography image is required to obtain a reliable visual scan and to compute reliable semiquantitative information. Dual display (B-mode US display and elastography display) should be preferred to maintain the scanning plane during the real-time acquisition of elastography images, especially if the method requires rhythmic pressure movements of the probe, which must be held perpendicular to the cylindrical bowel target (quasistatic method).

To reduce the variability of the method, a software spring indicator should show that the probe compression movements are correct. The elastography image must furthermore prove to be reproducible in subsequent tests to be used in clinical practice.

Since SE measures the relative stiffness of the tissues within the elastography region of interest (ROI) superimposed on a B-mode US image, the ROI should be large and include the target bowel at the center as well as the abdominal wall and the adjacent mesentery (Fig. 1a).

The dynamic range is preset with maximum visual impact on the elastography image. Using a moderately compressed color scale, the normal bowel walls are mainly green with shades of other colors (from turquoise and blue to yellow and red).

Fig. 1 SE oblique scan of the sigma. **a** The selected ROI on the anterior wall of the sigma (arrowhead) permits calculation of mean (*) and HRD (**) values. **b** The selected ROI on the anterior wall of the sigma (arrowhead) and reference ROI on the adjacent mesentery (arrow) permit calculation of ELX2/1 (1.77). **c** When the reference ROI is positioned on the abdominal-wall muscles (arrow), the value differs (ELX2/1=0.7)

In addition to probe frequency, the gain and transparency of the elastography image are crucial for obtaining the best visual results. The scanner software used in our institution permits the calculation of strain data obtained in a ROI selected on a target showing the data in a distribution curve called a histogram [5]. The results are shown in a graph where a “0–100” strain numerical scale is plotted on the horizontal axis (0 for maximum stiffness and 100 for maximum elasticity), while the number of pixels

related to the same strain value in the ROI are plotted on the vertical axis (Fig. 1a).

ROI drawing is an operator-dependent procedure. To reduce variability, we include the anterior visceral wall in the ROI, preferably in longitudinal scanning to the extent allowed by the elastographic image. If the affected intestinal tract is long, this procedure is usually repeated on adjacent segments, thus obtaining semiquantitative data reflecting the overall stiffness.

Among the descriptive numerical parameters of the distribution graph, “mean” (the mean value of all strain data measured in the selected ROI) and “% hardness 50” (HRD, the percentage of pixels in the ROI presenting a strain value < 50) seem to be significant in evaluating the affected bowel walls in Crohn’s disease (Fig. 1a).

Elaxto allows the calculation of the elasticity ratio (ELX2/1), that is, the ratio between the average of strain values measured in a reference tissue and the average of strain values measured in a target tissue (the bowel wall). As previously mentioned, ROI drawing is an operator-dependent procedure, and the elastic ratio involves positioning two ROIs. Furthermore, the reference ROI cannot be drawn on a normal bowel wall because of the small dimensions and peristaltic movements. Other tissues can be used as reference, such as the mesentery adjacent to the affected wall (Fig. 1b) or the abdominal muscle wall (Fig. 1c), with different results. If the mesentery in the SE image is not homogeneous, positioning the reference ROI may be difficult.

In Crohn’s disease, the elastography image shows some frequent patterns that can be classified into visual categories suggesting specific clinical conditions. In recent years, the classification published by Fufezan et al. [6] has been used in our institute. This classification system divides the results into three categories: **type A**, referring to a normal bowel or the remission of bowel disease with normal-bowel wall thickness and color pattern, mainly green (Fig. 2a); **type B**, referring to wall inflammation with thickened walls and the color stratification of the wall at least partially preserved (Fig. 2c–e) and **type C**, referring to bowel-wall fibrosis, where the color pattern is mainly turquoise and blue (Fig. 2f). The loss of bowel-wall color stratification appearing as a mix of several colors or as a homogeneous prevalence of a single color, usually blue, suggests structural abnormality and severe damage to the bowel wall.

Strain elastography using visual scan, mean, and HRD in the characterization of early Crohn’s disease

The onset of abdominal symptoms and the endoscopic detection of early elementary lesions characterize early Crohn’s disease. Aphthae and superficial serpiginous ulcers are

considered the earliest elementary pathological changes that may permit the endoscopic diagnosis of Crohn’s disease [7].

In the acute stage of the disease, ulcers may be deeper and sometimes confluent, and mucosal and submucosal edema may be more severe with cobblestone appearance (Fig. 3a, d). B-mode US shows increased bowel-wall thickness, and CD provides vascular signals (Fig. 3b). The SE visual scan usually classifies the damaged portion as **type B**, and semiquantitative parameters generally suggest only a slight to moderate increase in stiffness (Fig. 3c). However, US methods rarely detect superficial changes in the mucosa (aphthae and ulcers).

Severe mucosal edema encountered on endoscopy (Fig. 3d, ascending colon) may correlate with a marked increase in bowel-wall stiffness revealed by elastography semiquantitative parameters and visual-scan observations (Fig. 3f). In this case, the mean and HRD values may also suggest fibrotic processes (rather unlikely in this phase of the disease), but bowel-wall appearance at B-mode US and CD allows a correct interpretation of the elastography results.

Strain elastography using visual scan, mean, HRD and ELX2/1 in the characterization of long-term Crohn’s disease and complications (fistula and stenosis)

Crohn’s disease is characterized by chronic inflammation that increasingly affects the bowel wall, with the progressive alteration of the mucosal, submucosal, muscular, and serosal layers. The two most common long-term complications are fistulas and bowel strictures. Mucosal alterations are well documented at endoscopy, thanks to the presence of longitudinal ulcers, either parallel or variously oriented, and mucosal hyperemia and edema, possibly with cobblestone appearance [7].

US importantly contributes to documenting the extent and severity of the pathological process. In some cases, the characteristics of the affected visceral walls (deep ulcers, thickened muscular tunic, thickened mesentery, fistulas, stenosis) may suggest long-term disease (Table 1).

If the affected segment of the bowel is long, repeated B-mode US, CD, and elastography on the adjacent bowel segments may show inflammatory and fibrotic/degenerative phenomena scattered along the affected bowel wall.

Fistulas occur when the inflammatory exacerbation exceeds the limits of the bowel walls toward the adjacent mesentery, the adjacent bowels, the abdominal wall, and/or the retroperitoneal space. Infection and abscesses are possible complications in this clinical condition.

In Fig. 4, CD shows the site where acute inflammation has penetrated the serous layer with fistulization into the adjacent mesentery. The ileum wall is inhomogeneous with

Fig. 2 Classification of some frequent elastography patterns in Crohn's disease (Fufezan et al. 2015). **a Type A:** SE longitudinal scan of the terminal ileum. **b** Normal terminal ileum: endoscopic image of the same case as **a**. **c Type B:** SE longitudinal scan of the terminal ileum showing wall thickening with preserved color stratification and mainly blue–green–blue pattern representing the mucosal, submucosal, and muscular layers. **d Type B:** SE longitudinal scan

of the terminal ileum showing wall thickening with preserved color stratification and mainly blue/green–red/blue pattern representing the mucosal, submucosal, and muscular layers. **e Type B:** SE longitudinal scan of the terminal ileum showing wall thickening and a mainly green pattern with multiple shades of red and rare streaks of blue. **f Type C:** SE longitudinal scan of the terminal ileum showing wall thickening with a mainly blue pattern and shades of green

Fig. 3 Endoscopy, CD, semiquantitative parameters, and SE visual scan in one case of Crohn's disease affecting the terminal ileum and ascending colon. **a** Endoscopy of the terminal ileum. **b** CD oblique scan of the terminal ileum (arrowhead) shows slight thickening of the bowel wall with preserved stratification and vascular signals classified as grade 2 on the Limberg scale. **c** SE with semiquantitative parameters—mean 41 and HRD=65%—suggests a slight to moderate increase in stiffness; SE visual scan classifies this portion as **Type**

B. d Endoscopy of the ascending colon showing edematous mucosa with raised folds despite air insufflation (arrowheads) and multiple superficial serpiginous ulcers (arrow). **e** CD longitudinal scan of the ascending colon showing moderate thickening of the bowel walls with preserved stratification and vascular signals classified as grade 3 on the Limberg scale. **f** SE with semiquantitative parameters—mean 19 and HRD=96%—suggests marked increase in stiffness; SE visual scan classifies this bowel portion as **Type C** (mainly blue)

Table 1 Characteristic features of B-mode US, CD, and strain elastography images in early, long-term, and complicated Crohn's disease

	Early Crohn's disease	Long-term Crohn's disease	Complicated Crohn's disease
B-mode US	Bowel wall thickening + preserved wall echo- stratification or + poorly preserved wall echo- stratification	+ Diffuse thickening of the muscular and submucosal tunic + Deep ulcers + Thickening of the mesentery	+ Fistula + Abscess + Stenosis
CD	Detection of vascular signals	Detection of vascular signals	+ Increase in vascular signals adjacent to the fistula
SE visual scan	Usually Type B Rarely Type C	Type B or Type C (fibrosis, muscular hyperplasia, interstitial edema)	Type B or Type C (fibrosis, muscular hyperplasia, interstitial edema)
SE mean/HRD	Usually: slight-moderate increase in bowel wall stiffness Rarely: moderate-marked increase in bowel wall stiffness	From slight-moderate increase in bowel wall stiffness To moderate-marked increase in bowel wall stiffness +Possible increase in the stiffness of the mesentery	Usually: moderate or marked increase in bowel wall stiffness + Possible moderate or marked increase in the stiffness of the mesentery

partial stratification loss, and the fistula appears hypoechoic. Vascular signals are stronger at the fistula. SE semiquantitative parameters demonstrate a greater stiffness of the posterior wall adjacent to the fistula (mean 30, HRD = 87%) compared with the anterior wall (mean 37, HRD = 68%); an SE visual scan classifies both portions as **type B**. A careful visual observation permits the identification of harder blue areas (interstitial edema/fibrotic processes) in the posterior wall where the acute inflammatory process tends to invade the adjacent mesentery.

Stenosis is a complication in long-term Crohn's disease characterized by a reduced bowel lumen caliber and an altered bowel-wall structure, that usually is, stiffness caused by the prevalence of fibrotic scarring and irregular thickening of the submucosal and muscular layers.

B-mode US can accurately diagnose occlusive stenosis in the presence of reduced lumen caliber of the affected bowel and upstream lumen dilation. Furthermore, B-mode US and CD can suggest the inflammatory or fibro-cicatricial nature of the stenotic intestinal tract based on the thickness of the affected wall, the persistence or loss of wall stratification, and the presence or absence of vascular wall signals.

Quaia et al. have recently updated the elastography classification of bowel stenosis in Crohn's disease [8] and divided it into four main categories (**a** = homogeneous stiff wall, corresponding to Fufezan's **type C**; **b** = mainly blue–green/stiff stratified wall; **c** = mainly green–red/soft stratified wall; **d** = non-stratified wall with uneven appearance and color mix, green/blue if mainly stiff wall or green/red if mainly elastic wall).

In the case presented in Fig. 5, the walls of the obstructed ileum are only slightly thickened, and B-mode US shows preserved stratification. SE with semiquantitative values—mean 18, HRD > 95%, and ELX2/1 = 3.92—suggests marked ileal-wall stiffness (Fig. 5b, c), thus increasing diagnostic confidence. However, visual-scan elastography shows a predominantly green intestinal wall with irregularly distributed

blue areas; classification is, therefore, uncertain (Fufezan **type B**, Quaia **category d1**).

If B-mode US shows moderate to severe thickening of the obstructed bowel wall and CD vascular signals are classified as grade 3 on the Limberg scale (Fig. 6a, b), inflammation should be suspected.

The semiquantitative parameters—mean 37, HRD = 68%, and ELX2/1 = 2.04 (Fig. 6c, d)—suggest that the bowel wall is not as stiff as that of the previously described case (Fig. 5). An SE visual scan classifies the bowel walls as Fufezan **type B** or Quaia **category c** (preserved color stratification, green submucosal tunic with shades of red and mainly blue mucosa/muscular tunics).

Strain elastography using visual scan, mean, HRD, and ELX2/1 in the therapy and monitoring of Crohn's disease

In patients with Crohn's disease, the purpose of therapy is remission of the disease affecting the bowel wall as this is linked to a better prognosis (fewer hospitalizations because of exacerbation and fewer surgical interventions because of complications). The lesion site may be inaccessible to endoscopic procedures, and the disease usually affects the entire bowel wall. Diagnostic imaging (US, CT, MRI) is, therefore, useful in staging and monitoring the disease.

In the case presented in Fig. 7, B-mode US and CD show marked thickening of the ileum walls (> 9 mm), only partially preserved wall stratification, and vascular signals classified as grade 3 on the Limberg scale, thus suggesting exacerbation of the disease (Fig. 7b). The mean (53), HRD (43%), and ELX2/1 (0.93) values exclude a significant increase in bowel-wall stiffness, while an SE visual scan classifies the ileum appearance as **Type B** given the presence of red, brown, and yellow shades in the submucosa. A

Fig. 4 Crohn's disease affecting the ileum in the phase of exacerbation. **a** Contrast-enhanced CT enterography coronal scan shows two small inflammatory collections adjacent to the affected ileal loop (arrowheads). **b** CD longitudinal scan shows an inflammatory col (arrowheads). **c, d** SE with semi-quantitative computation of the posterior and anterior bowel walls

B-mode US follow-up confirms the regression of inflammatory phenomena showing normal bowel-wall thickness but with a mainly hyperechoic appearance.

In the case presented in Fig. 8, SE semi-quantitative measures (mean and HRD) suggest a progressive increase in stiffness of the affected ileum during a 16-month follow-up. At diagnosis after the first steroid therapy, the semi-quantitative values—mean 38 and HRD=67%—suggest only moderate

stiffness. After 12 months of biological therapy, the mean (31) and HRD (77%) values suggest moderate to severe stiffness. Four months later, in connection with the last exacerbation before surgery, the mean (23) and HRD (90%) values suggest severe stiffness. Surgery confirmed these data: a stiff ileum presenting wall thickening and a stiff mesentery (Fig. 8h).

By contrast, elastic ratio repeated at same-time intervals failed to document significant bowel-wall changes

Fig. 5 Crohn's recurrence on ileocolic anastomosis with episodes of partial obstruction. **a** Contrast-enhanced CT enterography coronal scan shows stenosis (arrow) and dilation of the bowel lumen upstream. **b, c** SE semiquantitative measurements confirm severe

increase in the stiffness of the stenotic portion. **d** Surgical findings confirm lumen stenosis, ileum wall thickening (arrows), normal mucosa upstream of the stenosis (arrowheads), and hypertrophy of the mesentery

(Fig. 8e–g; $ELX2/1 = 1.14/1.23/1.20$), probably because the concomitant progressive increase in stiffness of the adjacent mesentery was used as a reference target.

Visual observation SE classified the ileal segment as **Type B** during the entire 16-month follow-up as the different

patterns of blue shades in the bowel wall do not affect Fufezan's classification until the blue color is predominant.

In this case, an insufficient correlation among SE visual scan, Fufezan classification, elastic ratio, and mean and HRD values was evident.

Fig. 6 Crohn's disease affecting the last ileal loop diagnosed 10 years earlier: episode of bowel obstruction. **a** B-mode US longitudinal scan on the last ileal loop shows dilation > 3 cm* upstream of the stiff part of the terminal ileum, but the lumen is visible over about 5 cm*** before the stenosis. **b** CD of the ileal stenosis immediately distal to **a**

shows bowel-wall thickening and partial loss of stratification; vascular signals are classified as grade 3 on the Limberg scale. **c, d** Semi-quantitative measurements of the same segment suggest moderately increased stiffness

Discussion

SE is not routinely performed in clinical practice in the diagnosis and follow-up of gastrointestinal tract diseases. The reasons are numerous: the method for acquiring elastography images is different from the traditional real-time US method and requires a period of training, the interpretation of the images is not intuitive like B-mode US or color Doppler interpretation, and the results obtained with scanners using different elastography methods may not be comparable.

However, some elastography patterns—such as a blue pattern within the bowel wall from fibrotic scarring phenomena or increased interstitial edema and a red–yellow pattern

from the prevalence of inflammation—are easily interpreted. By contrast, elastography images characterized by other color patterns alternating in various ways within the bowel wall or combined with poorly preserved color stratification are difficult to interpret.

Visual observation of the elastography image is, therefore, an immediate method, but interpretation repeatability is poor if the appropriate classification systems are not adopted. Some classification systems and scores are proposed in the literature [6, 8–11], but no studies compare them, nor subsequent studies confirm their effectiveness (by authors other than the proponents). The numerous SE classification systems and scores indicate the difficulty

Fig. 7 Crohn's disease affecting the last ileal loop diagnosed 5 years earlier: exacerbation of abdominal symptoms. **a** Contrast MRI enterography sagittal scan: the affected ileal loop is evidenced in full with wall thickening and a layered appearance from mucosal and submucosal enhancement. **b** The same ileal segment as **a**; CD shows wall

thickening with vascular signals. **c, d** The same ileal segment as **b**; SE with semiquantitative parameters—mean, HRD, and ELX2/1—suggests only slightly increased stiffness. **e** B-mode US after 8 months of medical therapy shows normal thickness of the ileum walls, which appear echoic; the lumen is well depicted (arrow)

linked to the interpretation of the various elastography color patterns obtained in Crohn's disease.

Until 2015, the elastography classification system proposed by Havre et al. [9] was applied in our institution, although in our opinion, it is excessively complex. Later, the system proposed by Fufezan et al. [6] was adopted. It is simple but not exhaustive with regard to some elastography patterns encountered in clinical practice. The recently updated version proposed by Quaia et al. [8] is more exhaustive but limited to elastography patterns found in bowel stenosis.

To calculate semiquantitative values using the scanner software, US examination has to be interrupted to perform the desired processing, thus slowing down the procedure. As an alternative, data can be analyzed and reported later using a remote computer terminal.

Elastic ratio is a semiquantitative parameter used in clinical practice both in focal breast pathologies (reference tissue is the homogeneous adipose tissue) and in focal thyroid pathologies (reference tissue is the healthy thyroid parenchyma). In Crohn's disease, elastic ratio has been used in some studies, with the target ROI positioned on the intestinal wall and the reference ROI either on the mesentery or on the abdominal-wall muscles. Some authors claim that the most promising results using elastic ratio are obtained when the mesentery adjacent to the affected bowel is selected as reference tissue [12, 13]. However, other authors report unsatisfactory results with this method [14]. The mesentery can, to some extent, be influenced by Crohn's disease [11], thus altering the value of the elastic ratio (Fig. 8e–g).

In daily practice, we prefer adding the calculation of the mean and HRD parameters to the visual interpretation of the elastography images to summarize the distribution of strain values in the target ROI using a numerical value. In our opinion, the calculation of numerical values provides a more objective evaluation than visual interpretation and the possibility to compare bowel-wall stiffness measured in the course of the follow-up.

SE combined with mean-value calculation was evaluated in one study reported in the literature (an elastography system using a scale of 256 strain values) [15]. SE and mean-value calculation allowed the distinction between bowel-wall

fibrosis (mean 43 ± 25.9) and normal bowel walls (mean 169 ± 27.9) in ten patients with Crohn's disease and stenosis, which was subsequently resected. The software at our disposal (Elaxto using a scale of 100 strain values) assigns lower mean values to stenosis from fibrotic scarring (Fig. 5), but in proportion, they agree with those of the above-mentioned study.

In the literature are three recent reviews [16–18] that summarize the results obtained to date. They all emphasize that studies of larger patient populations and standardized methods are required to confirm the obtained results.

The recent EFSUMB guidelines (2017) further suggest elastography in evaluating pathological bowel-wall stiffness [19]. The level of evidence (4) and the degree of recommendation (C) reflect the need for further studies, but the broad consensus among experts (11/12) indicates the role that the method may soon acquire in monitoring these diseases.

Conclusion

Crohn's disease is a chronic inflammatory condition that causes progressive damage to the bowel wall, with possible evolution over time toward clinical complications such as fistula and stenosis formation.

Elastography does not provide differentiation from other intestinal pathologies [20] nor a histological diagnosis [9] but only information on bowel-wall stiffness, which can be correlated with wall damage because of the disease duration.

SE with visual observation of elastography color patterns representing the bowel wall is easily performed and, in some cases, provides relevant information.

In our opinion, elaborating semiquantitative parameters seems to provide more objective data than visual observation; it does not require classification systems and seems more appropriate for monitoring the evolution of the disease in clinical practice.

Funding This study was not funded by third parties.

Fig. 8 Crohn's disease affecting the distal ileum. **a** Contrast-enhanced CT at diagnosis shows the affected segment (arrow) adjacent to a small phlegm collection (arrowheads). **b–d** Subsequent SE with mean and HRD measurements suggests progressive increase in ileal-wall stiffness. **e–g** SE with ELX2/I measurements does not confirm increase in ileal-wall stiffness; SE visual scan classifies this portion as **Type B**. **h** Surgical findings show stenosis of the ileal lumen, marked thickening of the ileum wall (arrows), a deep ulcer (arrowhead), and hypertrophy of the mesentery

Compliance with ethical standards

Conflict of interest Andrea Giannetti, Marco Matergi, Marco Biscontri, Lucia Falconi, Francesco Tedone, Valeria Ussia, Luca Giovannelli, Luca Franci declare they have no conflict of interest related to this study.

Ethical standards All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki declaration of 1975, as revised in 2000.

Informed consent The patients provided written informed consent to the publication of information that could potentially lead to their identification.

Studies with human participants or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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