



# Imaging of intestinal fibrosis: current challenges and future methods

Ryan W Stidham and Peter DR Higgins

## Abstract

Crohn's disease (CD) activity assessments are dominated by inflammatory changes without discrete measurement of the coexisting fibrotic contribution to total bowel damage. Intestinal fibrosis impacts the development of severe structural complications and the overall natural history of CD. Measuring intestinal fibrosis is challenging and existing methods of disease assessment are unable to reliably distinguish fibrosis from inflammation. Both the immediate clinical need to measure fibrosis for therapeutic decision-making and the near-future need for tools to assess pipeline anti-fibrotic medications highlight the demand for biomarkers of fibrosis in CD. Developing non-invasive technologies exploit changes in intestinal perfusion, mechanical properties, and macromolecular content to provide quantitative markers of fibrosis. In this review of existing and experimental technologies for imaging intestinal fibrosis, we discuss the expanding capabilities of quantitative MR and ultrasound imaging, encouraging developments in non-invasive elastography, and emerging novel methods including photoacoustic imaging.

## Keyword

Crohn's disease; intestinal strictures; intestinal fibrosis; disease activity; elasticity imaging; magnetic resonance enterography

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## Introduction

Crohn's disease (CD) is classically considered an idiopathic illness of intermittent segmental intestinal inflammation. Transmural bowel wall injury is also impacted by wound healing mechanisms which result in the development of cumulative and irreversible intestinal fibrosis. Over time, inflammation and fibrosis together cause progressive bowel wall thickening, stricture development, and obstructing and penetrating complications.<sup>1</sup> An argument could be made that intestinal fibrosis is most responsible for a complicated and disabling CD course, considering that despite the introduction of anti-tumor necrosis factor alpha (TNF) therapies to control inflammation, surgical rates have not changed in nearly 20 years in the USA.<sup>2</sup> The complexity of CD management decisions are often related to our inability to determine the relative contribution of inflammation and fibrosis in a damaged bowel segment. The degree of intestinal fibrosis present underlies common clinical questions for individual patients: What is the appropriate therapeutic intensity at diagnosis? Is the current therapeutic

regimen sufficient to prevent future structural complications? Will increasing therapeutic intensity in the setting of existing stricturing disease be sufficient to prevent surgery? This review will discuss existing, emerging, and experimental imaging methods to detect and measure intestinal fibrosis in CD.

## The challenges of assessing intestinal fibrosis

Because of the inability of ileocolonoscopy with biopsy to interrogate transmural injury, cross-sectional imaging methods, including ultrasound (US), computed tomography enterography (CTE), and magnetic resonance enterography (MRE), have become essential companions to endoscopy.<sup>3,4</sup> Excellent image-based disease

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Division of Gastroenterology and Hepatology, Department of Medicine, University of Michigan Health System, Ann Arbor, MI, USA

### Corresponding author:

Ryan W Stidham, Division of Gastroenterology and Hepatology, Department of Medicine, University of Michigan Health System, 1500 East Medical Center Drive, 3912 Taubman Center, Ann Arbor, MI 48109, USA.  
Email: ryanstid@med.umich.edu

activity assessments, including the magnetic resonance index of activity (MaRIA), are strongly correlated with endoscopic activity, but principally score inflammatory factors.<sup>5</sup> Yet, the concept that a high or low degree of bowel wall contrast enhancement is characteristic of predominantly inflammatory or fibrotic intestinal disease has been questioned. Adler and colleagues, along with other groups, have showed that that the radiologist global impression of whether a stricture was “active” or “inactive” was not associated with the presence or degree of fibrosis.<sup>6,7</sup> The Lémann index represents an international effort to more comprehensively describe total bowel damage accounting for stricturing and penetrating disease using imaging.<sup>8</sup> However, obvious strictures represent late stage macroscopic events, when the window for medical intervention is nearing closure. Further, claims that bowel dilation is associated with the degree of intestinal fibrosis remain controversial.<sup>9</sup> One could make a pragmatic argument that treating to an endpoint of objective resolution of inflammation to halt the progression of fibrosis is sufficient. However, emerging data raises the possibility that while inflammation may initiate fibrogenesis, for poorly understood reasons, fibrosis could auto-propagate even after inflammation is extinguished.<sup>10</sup> Taken together, these points capture the need to objectively measure intestinal fibrosis. Given our inability to safely and routinely obtain full thickness intestinal samples, several methods are being explored to provide a “virtual biopsy,” relying on changes in tissue perfusion, mechanics, and macromolecular composition as surrogate biomarkers of fibrosis.

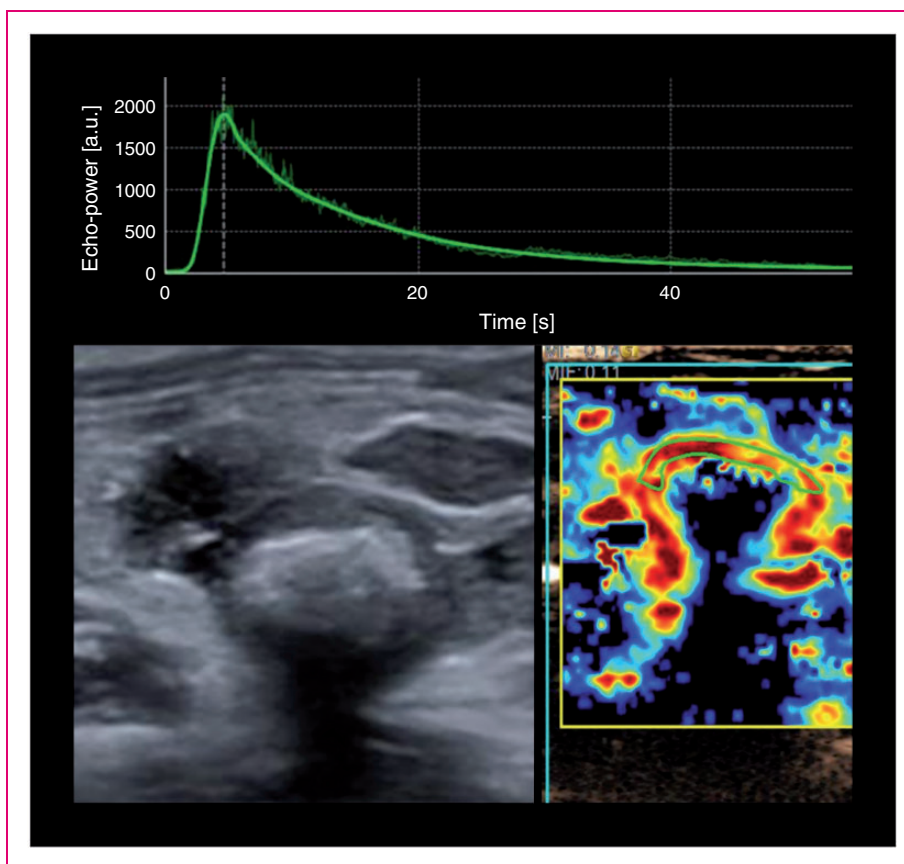
### Perfusion as a surrogate for intestinal fibrosis

The neovascularization and changes in vascular auto-regulation observed in intestine affected by CD have been studied as methods to grade histologic inflammation.<sup>11</sup> Fibrotic histologic changes are hypothesized to retard blood flow. Careful quantitation of perfusion dynamics using traditional MRE methods has yielded encouraging data supporting its use as a marker of fibrosis. Comparing MRE to graded histology in 41 resected specimens, Rimola and colleagues found that imaging findings of inflammation, including high T2 signal, mucosal hyper-enhancement, and the presence of ulcerations, were associated with the histologic grade of inflammation ( $p=0.02$ ,  $0.03$ ,  $<0.01$ , respectively) but not the grade of fibrosis ( $p=0.57$ ,  $0.60$ ,  $0.85$ , respectively).<sup>12,13</sup> Alternatively, delayed progressive enhancement over a 7-minute observation period separated low- and high-grade fibrosis (14% vs. 35% enhancement gain,  $p<0.01$ ) but not inflammation. The authors also show that using high T2 signal

intensity to grade inflammation and delayed progressive enhancement to grade fibrosis can together coarsely classify intestine histology, moderately agreeing with paired tissue sample analysis ( $\kappa=0.59$ , 95% CI 0.40–0.77).

An alternative method of assessing tissue inflammation, diffusion weighted imaging (DWI), has also been studied as a quantitative method of assessing inflammation and may provide a method to distinguish inflammation from fibrosis.<sup>14</sup> In a small study of 28 CD patients undergoing elective surgical resection, the mean apparent diffusion coefficient (ADC) of fibrotic vs. non-fibrotic tissue was 2282 (1998–2772) and 1714 (1433–2311), respectively,  $p=0.023$ .<sup>15</sup> The linear correlation between ADC and the degree of inflammation by histology was not statistically significant ( $p=0.090$ ), though a trend of decreasing ADC with increasing inflammation score was observed. At this juncture it is unclear whether DWI can discriminate intestinal fibrosis from inflammation sufficiently enough to be clinically meaningful. The most notable limitation of these and other studies investigating fibrosis biomarkers is that the requirement for full thickness histology limits subjects to those undergoing surgery for intractable disease. Study samples are therefore biased towards moderate to severe fibrosis and few specimens contain intestinal damage dominated by inflammation; only 3/41 subjects had grade 1 fibrosis in the Rimola et al. study. The lack of access to full thickness histology, over a spectrum of disease durations and severities, remains a key hurdle in intestinal fibrosis research.

Tissue perfusion characteristics can also be assessed by ultrasound. Contrast-enhanced ultrasound (CE-US) uses intravenously administered microbubbles which can be quantified and standardized using image analysis platforms (Figure 1).<sup>16</sup> Readouts can provide not only peak contrast values, but also tissue perfusion kinetics; this is a more objective and quantitative method than traditional Doppler US (Figure 1). A 14 patient study of CE-US showed that delayed contrast wash-in ( $p=0.02$ ) and wash-out ( $p=0.008$ ) were associated with therapeutic non-response, though no full thickness histology was available.<sup>17</sup> A 39 subject study where patients were dichotomized as being predominantly fibrotic (requiring elective surgery for refractory stricturing disease), or inflammatory (no strictures, but required steroid or anti-TNF), found those judged to be fibrotic had a slower rate of perfusion compared to the inflammatory group (22.6 vs. 45.3 mL/min per 100 mL,  $p=0.003$ ).<sup>18</sup> The same group found that the ratio of intestinal tissue perfusion to bowel wall thickness (cutoff of 0.56 mL/min/mm) predicted surgical vs. medically managed disease with an AUROC of 0.92 and sensitivity and specificity 82% and 94%, respectively.



**Figure 1.** Contrast enhanced ultrasound (CE-US) has demonstrated some promise in providing better measurement of intestinal inflammation based on quantitation of perfusion characteristics. Perfusion kinetics, including inflow, outflow, and flow rate, can all be assessed within an area of interest. CE-US is being explored as a means to separate inflammation from fibrosis in Crohn's disease.

These CE-US studies presented are subject to error stemming from using clinical decisions, outcomes, and unverified gold standards as surrogates of fibrosis. It is also possible that the imaging findings influenced clinical decision making, biasing the results toward reporting an inflated accuracy. Though longitudinal studies and more histopathology correlates are needed, tissue perfusion characteristics quantified by MRE and CE-US appear correlated with intestinal fibrosis.

### Tissue elasticity as a surrogate for intestinal fibrosis

The excess deposition of extracellular matrix along with disorganized smooth muscle proliferation together contribute to changes in the mechanical properties of fibrostenotic intestinal damage. Direct measurement of intestine elasticity in animal models and *ex-vivo* human CD intestinal specimens have shown the degree of fibrosis is associated the degree of tissue stiffness.<sup>19</sup> This correlation has drawn attention to the use of non-invasive stiffness measurement as a

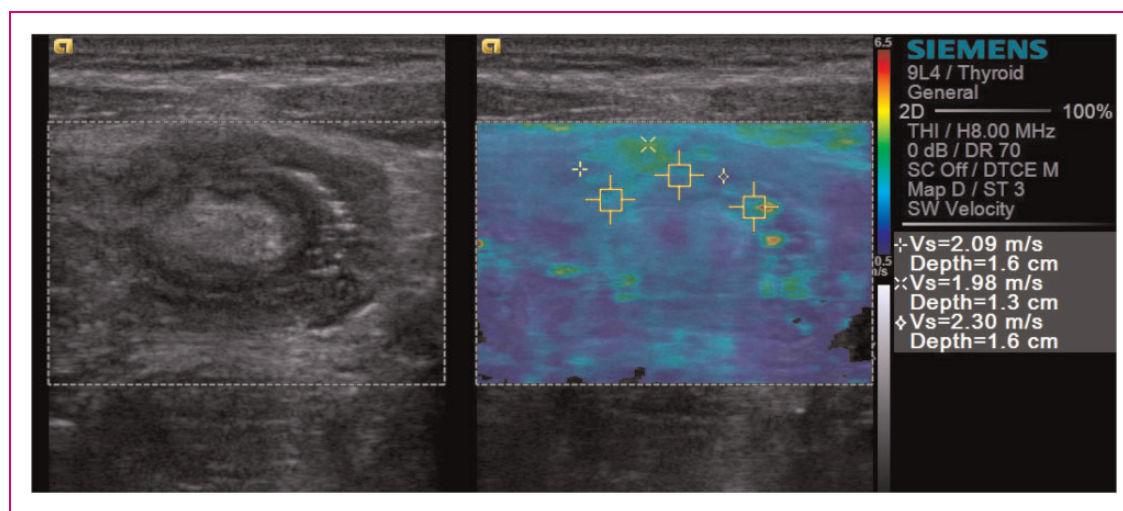
surrogate marker of fibrosis. Ultrasound strain imaging quantifies the 'hardness' or 'softness' of a tissue as a function of tissue compressibility. Using a two-dimensional speckle tracking technique for quantitation of tissue distensibility, non-invasive strain assessment strongly correlated with direct stiffness measurements of intestine.<sup>20</sup> Using the TNBS rat model of intestinal injury, ultrasound strain imaging was able to distinguish inflamed from fibrotic-inflammatory tissue (2.07 vs. 1.10,  $p=0.037$ ) and also correlated with the presence of fibrosis in a small human pilot ( $\rho=0.81$ ,  $p=0.008$ ). An important limitation of this bowel elastography technique was that it does not produce real time results, requiring extensive post-study image processing.

New approaches to elastography are being investigated to determine their ability to measure intestinal fibrosis and predict therapeutic responsiveness in CD. In a small study of 10 CD patients undergoing intestinal resection, real-time ultrasound elastography (US-RTE) was shown to be associated with the degree of muscularis thickness ( $p=0.006$ ), Masson's trichrome stain scores (4 vs. 0,  $p<0.001$ ), and

quantitation of high vs. low collagen content by western blot (2.01 vs. 0.87,  $p=0.009$ ).<sup>21</sup> US-RTE offers advantages of immediate bedside results and standardization of applied freehand force by using a pressure-sensitive transducer. Shear wave elastography (SWE) is an alternative technique that generates an ultrasonic pressure wave (using acoustic radiation force impulse) which can be focused on a specific region of bowel wall. The resulting orthogonal shear wave speed through the tissue can be measured; increasing speed is correlated with increasing tissue density and stiffness (Figure 2). SWE has also demonstrated an ability to distinguish fibrotic and inflammatory intestinal damage in animal models with an AUROC of 0.971, exhibiting a PPV and NPV of 95.0% and 92.9%.<sup>22</sup> In addition, shear wave speed has been shown to modestly correlate with the histologic grade of fibrosis ( $\rho=0.60$ ,  $p=0.01$ ), but not inflammation ( $\rho=0.24$ ,  $p=0.36$ ) in *ex-vivo* human intestinal specimens.<sup>23</sup> A recent study of 23 patients undergoing surgical resection found elastography able to distinguish severe fibrosis from inflammation, though it was unable to separate mild-moderate histologic fibrosis from predominantly inflammatory disease.<sup>24</sup> Much more study is needed prior to incorporating elastography into routine clinical care, including establishing reproducibility, dynamic range, the ability to quantify intermediate gradations of fibrosis, and ultimately predict natural history and clinical outcomes in CD.

### PET and metabolic imaging for intestinal fibrosis

Positron emission tomography (PET), commonplace in oncologic follow-up, has been investigated as a method to differentiate predominantly inflammatory from fibrostenotic bowel damage. Fluorodeoxyglucose (FDG)-PET, combined with CT or MR for anatomic localization, has been explored under the hypothesis that inflammatory tissue will have increased metabolic activity compared to fibrotic bowel. Small pilot studies to date have demonstrated mixed, generally discouraging, results for PET-imaging to distinguish inflammation from fibrosis. A recent study showed that PET-MR in 19 patients undergoing elective bowel resection was able to predict the histology of resected segments (fibrosis alone vs. a mixed fibro-inflammatory disease), albeit with underwhelming diagnostic accuracy (sensitivity 0.67, specificity 0.73).<sup>25</sup> Lenze and colleagues prospectively evaluated PET-CT, MR-enteroclysis, and B-mode ultrasound for characterizing stricturing disease and predicting therapeutic outcomes. While non-significant trends in their data suggested some ability of all modalities to predicting medical response vs. surgical management within 6 months, no single technique was superior.<sup>26</sup> Considering the cost, radiation exposure, and marginal performance, traditional metabolic imaging by FDG-PET has not demonstrated the potential for fibrosis detection relative to other modalities.



**Figure 2.** Ultrasound stiffness imaging using shear wave speed measurement. In shear wave imaging, an ultrasonic pulse is directed at a region of interest, generating a shear wave within the tissue that can be measured by the transducer. A ileal stricture is shown by traditional B-mode scan (left) with real-time quantitation of shear wave speed within the scan field (right). Shear wave speed is correlated to tissue stiffness, which itself is associated with the degree of fibrosis.



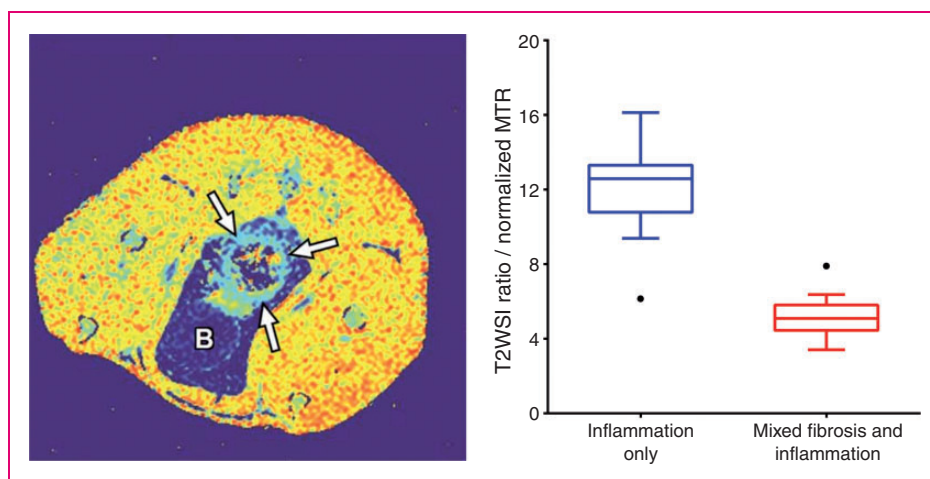
### Experimental techniques: direct macromolecule detection

Methods discussed thus far have explored surrogates of fibrosis which remain susceptible to variations in coexistent inflammation. Exciting new directions in macromolecule detection, specifically collagen, offer a more direct measurement of fibrosis. Magnetization transfer MR imaging (MT-MRI) is an alternative contrast technique that detects the exchange of protons (magnetization) between fixed macromolecules and surrounding free water within a tissue.<sup>27</sup> In this way, MT-MRI can detect collagen and other large macromolecules. Adler and colleagues showed that MT-MRI was able to quantify fibrosis and distinguish fibrosis from inflammation in an animal model of intestinal injury, with a PPV and NPV of 92% and 83%.<sup>28</sup> MT-MRI correlated well with both histologic scoring ( $\rho=0.74$ ) and collagen quantitation ( $\rho=0.72$ ). Subsequent studies improved the histologic discrimination of MT-MRI by adding a marker of inflammation, T2 weighted signal intensity, with a resulting AUROC of 0.98 for detecting bowel wall fibrosis in the TNBS rat model of intestinal damage (Figure 3).<sup>29</sup> Pilot studies have demonstrated the feasibility of MT-MRI in humans for separation of predominantly inflammatory and fibrotic intestinal damage.<sup>30</sup> In line with the limitations of fibrosis research discussed, these human pilots use surrogate endpoints themselves (radiologist definition of fibrotic vs. inflammatory). Within these limitations, MT-MRI, a technique available with existing hardware that incurs no

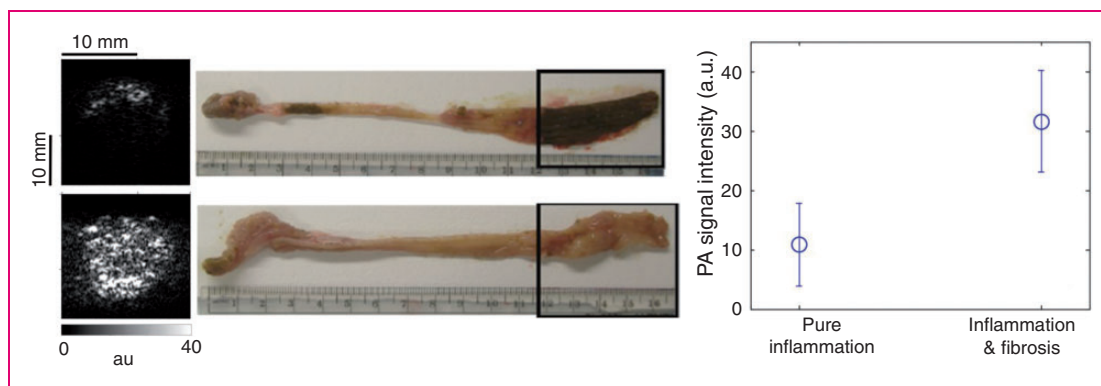
additional cost, provides a novel method for fibrosis measurement in CD.

Another experimental method gaining attention for molecular quantitation is photoacoustic imaging (PAI). PAI uses pulsed laser light to penetrate tissues at variable depths. The resulting molecular vibrations produced in the megahertz range can be detected by ultrasound for macromolecular signatures and two- or three-dimensional image production.<sup>31</sup> Exploiting the threefold difference in the optical absorption of hemoglobin (a molecular proxy for blood flow and inflammation) and collagen (a molecular proxy for fibrosis) at 532 nm and 1370 nm, PAI may provide a means to contrast hemoglobin and collagen from surrounding macromolecules and thus quantify fibrosis. Using the TNBS-rat model of intestinal injury, PAI at 1370 nm exhibited a 2.9-fold increase in average pixel intensity within mixed fibro-inflammatory intestinal segments compared to pure inflammatory injury,  $p < 0.0001$  (Figure 4).<sup>32</sup> Several technical challenges remain, including adjusting for the optical absorption of water (near 1300 nm), and a more efficient means of laser light delivery with tandem ultrasound. Human studies of PAI in CD are planned, using endoscopically delivered laser light combined with transabdominal and endoscopic ultrasound.

Endomicroscopy with molecular tagging is an exciting imaging method on the horizon with potential applications in CD. Atreya and colleagues recently showed the ability to perform *in vivo* membrane-bound TNF measurement using fluorescent antibodies and real-time endoscopic confocal laser microscopy.<sup>33</sup>



**Figure 3.** Magnetization transfer (MT) MRI for quantifying fibrosis. MT-MRI detects heavy macromolecules within a tissue, including collagen. In the TNBS-rat model of intestinal inflammation and fibrosis, a parametric map of calculated MT-ratios shows the bowel (arrows) having less MT effect than surrounding muscle, but more than fat and fluid in the bladder (B). The relationship between T2-weighted signal intensity and MT-ratio (left) provides good discrimination between inflammatory and mixed fibro-inflammatory disease of the bowel wall in this animal model.<sup>29</sup>



**Figure 4.** Photoacoustic imaging (PAI) for fibrosis in an animal model of Crohn's disease. Relying on the differential optical absorption spectra of collagen and hemoglobin, PAI may separate inflammation from fibrosis. In the rat-TNBS model of intestinal damage, PA signal from the bowel at 1370 nm is higher in mixed fibro-inflammatory compared to inflammation alone.<sup>32</sup>

In 25 subjects, anti-TNF responders had a mean of 30 TNF+ cells per field compared to 11 TNF+ cells in non-responders ( $p = 0.0004$ ). While molecular tagging and real-time confocal histology is presently restricted to the mucosa and submucosa, molecular tagging within the bowel has been accomplished in IBD. Apoptosis was assessed in animal models and 14 humans using IV administration of technetium-99m-annexin V followed by CT-scintigraphy prior to anti-TNF treatment.<sup>34</sup> Subjects with anti-TNF response exhibited 98.7% bowel uptake of <sup>99m</sup>Tc-annexin V in affected segments compared to 15.2% in non-responders. Radiolabeling techniques could conceivably be applied to constituents of fibrosis. Finally, emerging nanotechnology is providing additional tools for intestinal assessment. Nanoparticles can be engineered for high-affinity tissue or molecular binding, can be detected by multiple non-ionizing methods, and are stable enough for both intravenous and oral administration.<sup>35</sup> Orally administered nanoparticles have been used as a photoacoustic contrast agent to improve bowel motility assessments, with the potential for additional sophisticated intestinal imaging applications.<sup>36</sup>

Concepts of CD activity are evolving from isolated objective measures of inflammation to current efforts to objectively measure cumulative total bowel damage. Accurate and sufficiently dynamic measures of intestinal fibrosis will have an immediate impact in our understanding of the natural history of CD and an individual's prognosis. Objective fibrosis measures will help guide decisions regarding both medical vs. surgical treatment, as well as the intensity of therapy needed to avoid disease complications. Finally, we are on the cusp of anti-fibrotic therapies. Pirfenidone and nintedanib, small molecules that inhibit or downregulate key mechanisms of fibrosis, are now approved by the EMA and FDA for the

treatment of idiopathic pulmonary fibrosis. The bowel imaging techniques presented will be needed to evaluate the efficacy of candidate anti-fibrotic therapies in CD. Independent measurements of inflammation and fibrosis will together provide a more comprehensive description of intestinal damage for increasingly precise and individualized treatment of CD.

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#### Conflict of interest

The authors have no potential conflicts of interest relevant to this manuscript to declare.

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