Chapter 11
Histopathology of Intestinal Fibrosis

Ilyssa O. Gordon

Abstract Fibrostenotic inflammatory bowel disease classically refers to Crohn's disease, where stricture and submucosal fibrosis are frequent and defining histopathologic features. Descriptions of histologic fibrosis in ulcerative colitis have existed for over half a century, but are not currently part of the clinical diagnostic framework. Histologic scoring systems for fibrosis in inflammatory bowel disease are varied and highlight the need for improved histopathologic correlation, given recent advances in our understanding of the pathophysiology of intestinal fibrosis.

Keywords Fibrosis · Histopathology · Muscularis mucosae · Submucosa · Stricture Inflammatory bowel disease · Histologic fibrosis score

11.1 Histopathology of Fibrosis in Crohn's Disease

Submucosal fibrosis is a pathologic hallmark of Crohn's disease. Normal submucosal collagen and adipose tissue are replaced by fibrous tissue which contracts the submucosal area (Fig. 11.1). Strictures are areas of marked submucosal fibrosis, along with hyperplasia of the muscularis mucosae, which can become so thick as to obliterate the submucosa [1] (Fig. 11.2). Expansion of the muscularis mucosae, including hyperplasia, architectural disarray, and collagen deposition, accounts for about half of the increased wall thickness of an ileal stricture in Crohn's disease [2]. Strictures also often contain hypertrophic nerves (Fig. 11.3). Submucosal arteries and veins often have fibromuscular hyperplasia in strictured areas [2] (Fig. 11.4). Deeper in the bowel wall, muscularis propria hypertrophy can be seen, and along with disorganization and fibrosis, leads to an overall thickened muscularis propria, although this feature is not a diagnostic hallmark [3]. On gross examination, creeping fat or fat wrapping, is a common finding and a major pathologic feature, characterized by fat extending along the antimesenteric border.

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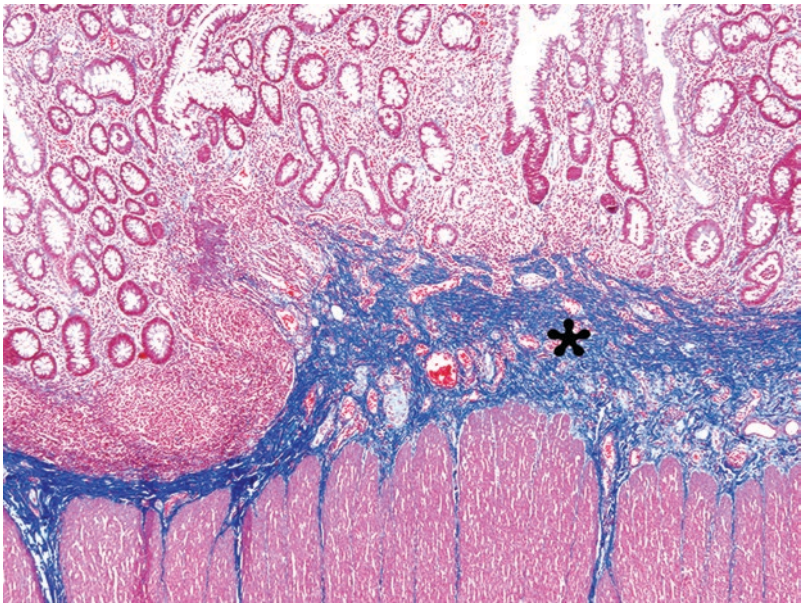

Fig. 11.1 Submucosal fibrosis in Crohn's disease. Fibrous tissue is present within the submucosal area (black star) (Masson Trichrome, original magnification 4×)

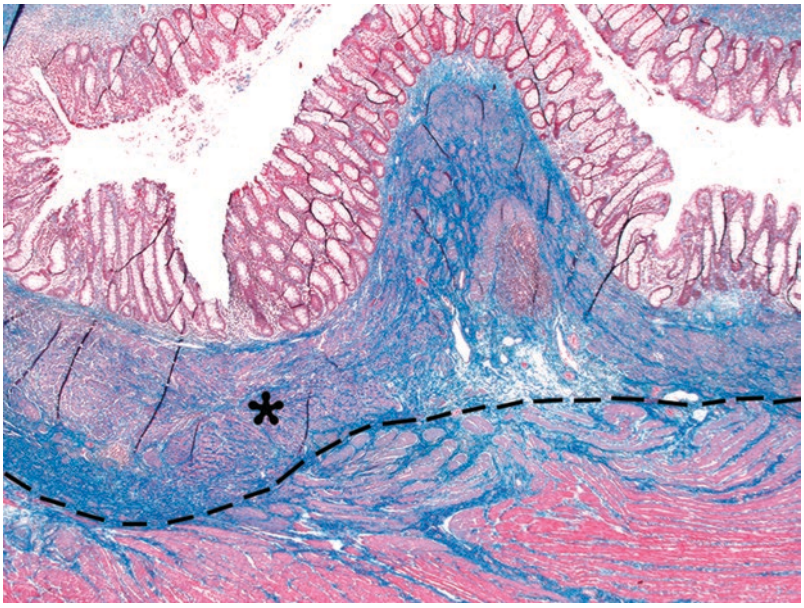

Fig. 11.2 Stricture in Crohn's disease. In addition to submucosal fibrosis, marked hyperplasia of the muscularis mucosae (black star) can obliterate the submucosal area. The dotted line demarcates the submucosa above from the muscularis propria below. (Masson Trichrome, original magnification 4×)

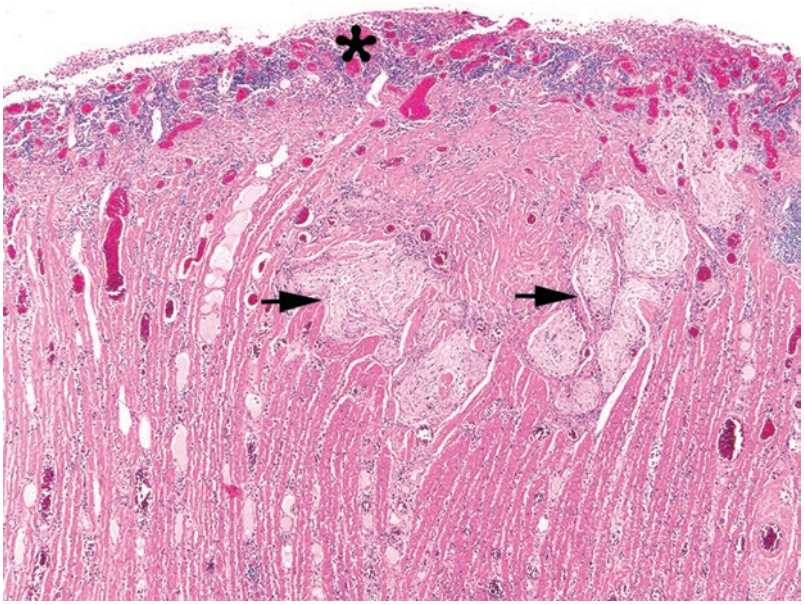

Fig. 11.3 Stricture in Crohn's disease. Hypertrophic nerves (black arrows) are present in the submucosa of this ulcerated stricture (black star indicates ulcerated luminal surface) (Hematoxylin & Eosin, original magnification 4×)

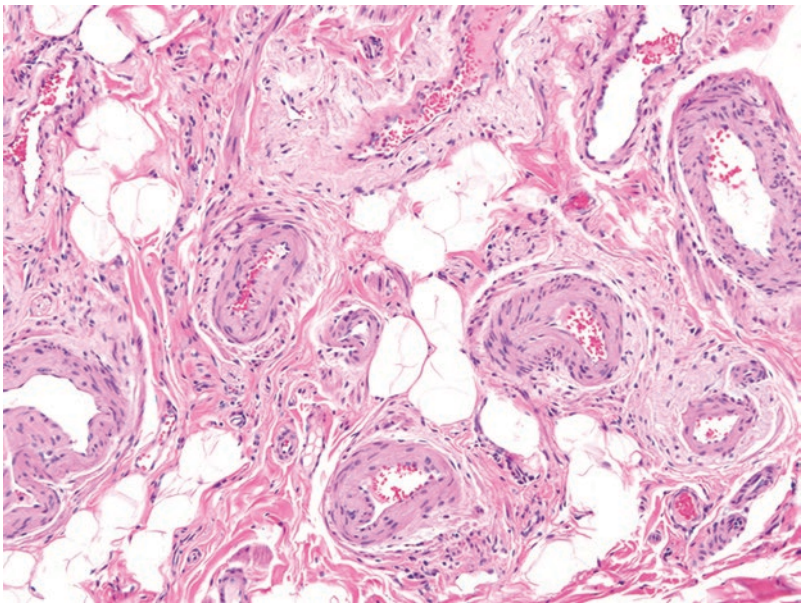

Fig. 11.4 Stricture in Crohn's disease. Fibromuscular hyperplasia of submucosal arteries and veins (Hematoxylin & Eosin, original magnification 10×)

It is not known whether hyperplasia of the muscularis mucosae and nerves occur as a result of a prolific response following ulcerating injury (i.e. aberrant wound healing [4]) or if they develop in response to other factors, such as mesenchymal growth factors, related to inflammation or gut bacteria; although a combination of these factors is most likely involved [5–7]. For example, intestinal fibroblasts express Toll-like receptor-4, which is acted upon by bacterial lipopolysaccharide (LPS) to activate a nuclear factor kappa B pathway, resulting in collagen contraction [7]. LPS also enhances connective tissue growth factor expression by decreasing expression of the transforming growth factor-beta inhibitor, smad-7 [7]. Other TLRs, including TLR-9, as well as chemokines, such as CXCL8, and cytokines also play a role in mesenchymal-bacterial interactions at the molecular level [6].

11.2 Histopathology of Fibrosis in Ulcerative Colitis

Standard descriptions used in diagnostic pathology do not include fibrosis as a histologic feature of ulcerative colitis. Furthermore, as ulcerative colitis is defined as having pathologic features restricted to the mucosa and superficial submucosa, the presence of a stricture in UC is immediately concerning for an infiltrative carcinoma, rather than a benign process. Looking to the published literature, however, 71% to 100% of all clinically detected strictures in UC patients were benign [8–10]. The risk of a stricture being due to malignancy is associated with longer duration of disease and location of the stricture, with rectal strictures being more common (68%), and therefore more often benign (90%), and strictures of the right colon being malignant 87–100% of the time [8, 11].

Histologic studies of benign strictures in UC have of necessity been performed on resection specimens. Goulston et al. compared the thickness of the muscularis mucosae and inner layer of muscularis propria in benign strictured and non-strictured UC areas, and found 40-fold and 20-fold thickening, respectively, as compared to non-strictured UC controls; concluding that fibrosis alone was insufficient to explain the stricture [11]. Other studies have described fibrosis along with muscular hypertrophy, including marked submucosal fibrosis in 20 of 28 benign UC strictures [12]. Microscopic examination of benign UC strictures has also revealed expansion of the submucosa by fat, which may be a factor contributing to luminal narrowing [13].

Descriptions of fibrosis in UC outside of the presence of strictures is also lacking [14]. In a study of UC proctocolectomy resections with dysplasia in an American center as compared to a Japanese center, lamina propria fibrosis in non-dysplastic areas was more prominent in American cases as compared to Japanese cases [15]. Interestingly, in control cases without dysplasia, there was no difference in lamina propria fibrosis in the two populations [15]. The authors suggested longer disease duration in the American group and differences in medications as possible explanations for the findings [15]. One very early study from 1949 describes fibrosis in the wall of UC resections in the context of extensive ulceration [16]. This concept was

also discussed in the 1950s and 1960s by Lumb et al. [17, 18]. Indeed, in the wound repair process after epithelial injury, including gut epithelial injury, it is generally accepted that fibrosis is part of the post-inflammatory organization of granulation tissue [19, 20].

We described histologic patterns of lamina propria fibrosis and muscularis mucosae alterations in non-strictured UC resections, and found these histologic features to be correlated with prior medication use and inflammatory activity [21]. The most striking pattern of lamina propria fibrosis is a band of fibrosis between the base of the crypts and the muscularis mucosae, essentially replacing the more typical basal lymphoplasmacytosis (Fig. 11.5). Similar to other studies finding alterations and thickening of the muscularis mucosae in UC, we have also seen altered muscularis mucosae with patterns of splaying, usually with interspersed fibrosis (Fig. 11.6), as well as thickening of the muscularis mucosae, and finally splitting and even duplication of the inner and outer layers of muscularis mucosae (Fig. 11.7).

Regarding submucosal fibrosis in non-strictured UC resections, we observe this phenomenon in ulcerated areas (Fig. 11.8) as well as in non-ulcerated areas. In non-ulcerated areas, fibrous bands within the submucosa typically form adjacent to the muscularis mucosae and muscularis propria and are otherwise perpendicular to the luminal flow (Fig. 11.9). Submucosal fibrosis can be identified in diagnostic H&E

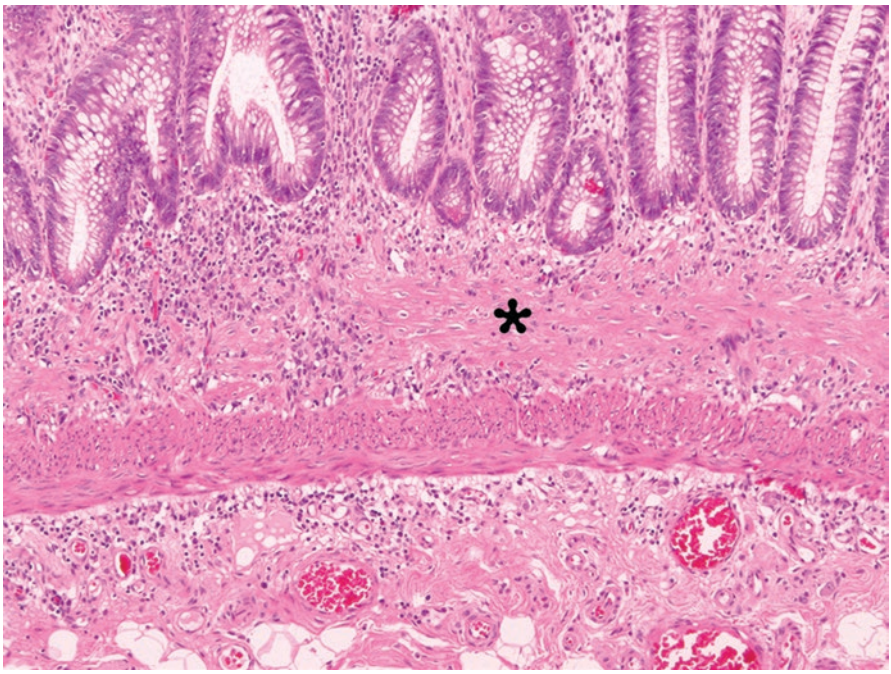

Fig. 11.5 Lamina propria fibrosis in ulcerative colitis. A band of fibrosis (black star) between the base of the crypts and the muscularis mucosae replaces the typical basal lymphoplasmacytosis (Hematoxylin & Eosin, original magnification 10×)

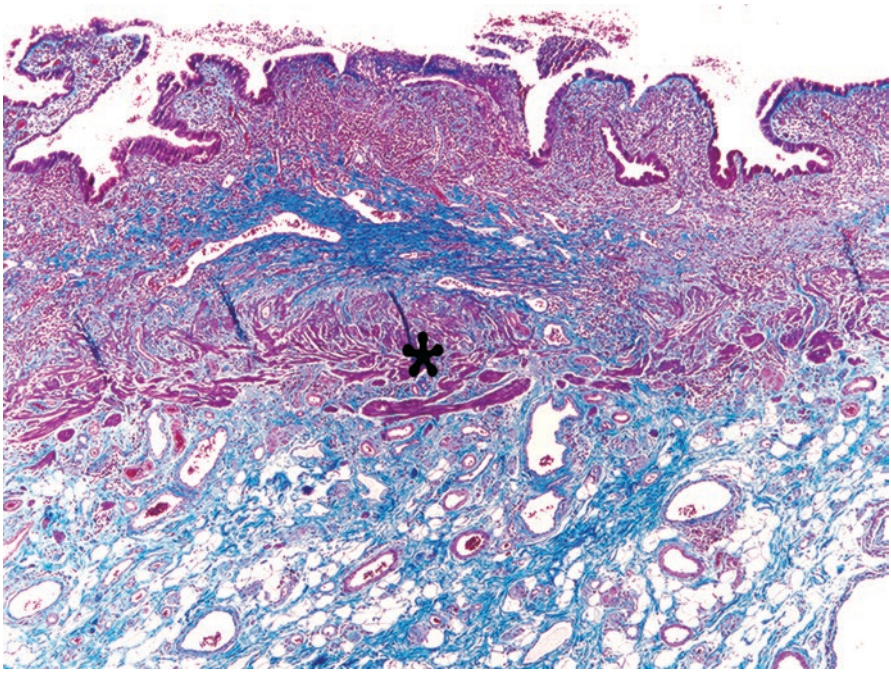

Fig. 11.6 Muscularis mucosae alterations in ulcerative colitis. Hyperplastic and splayed muscularis mucosae (black star) with interspersed fibrosis (blue) (Masson Trichrome, original magnification 4×)

stained sections without the aid of a trichrome stain, and the degree of submucosal fibrosis is associated with the severity of intestinal inflammation [22]. Apart from fibrosis associated with deep mucosal ulceration in fulminant disease, significant changes in the muscularis propria are not typically seen.

11.3 Pathology Fibrosis Scoring Systems in Inflammatory Bowel Disease

Fibrosis scoring systems in inflammatory bowel disease are typically based on imaging and biomarkers [23]. Histologic scoring of fibrosis in Crohn's disease originated from studies in rodents [24, 25], as well as human studies comparing radiographic findings with resection specimen findings [26–29], and early studies examining resection specimen margins to determine factors associated with recurrent disease [30, 31].

The earlier rat scoring system by Theiss et al. (Table 11.1) is progressive and cumulative, and is based on evaluation of sections stained with Masson trichrome and Sirius red [24]. Progression starts from submucosal collagen deposition (score 1), and adds on mucosal collagen deposition (score 2), muscularis mucosae collagen deposition and disorganization (score 3), muscularis propria collagen

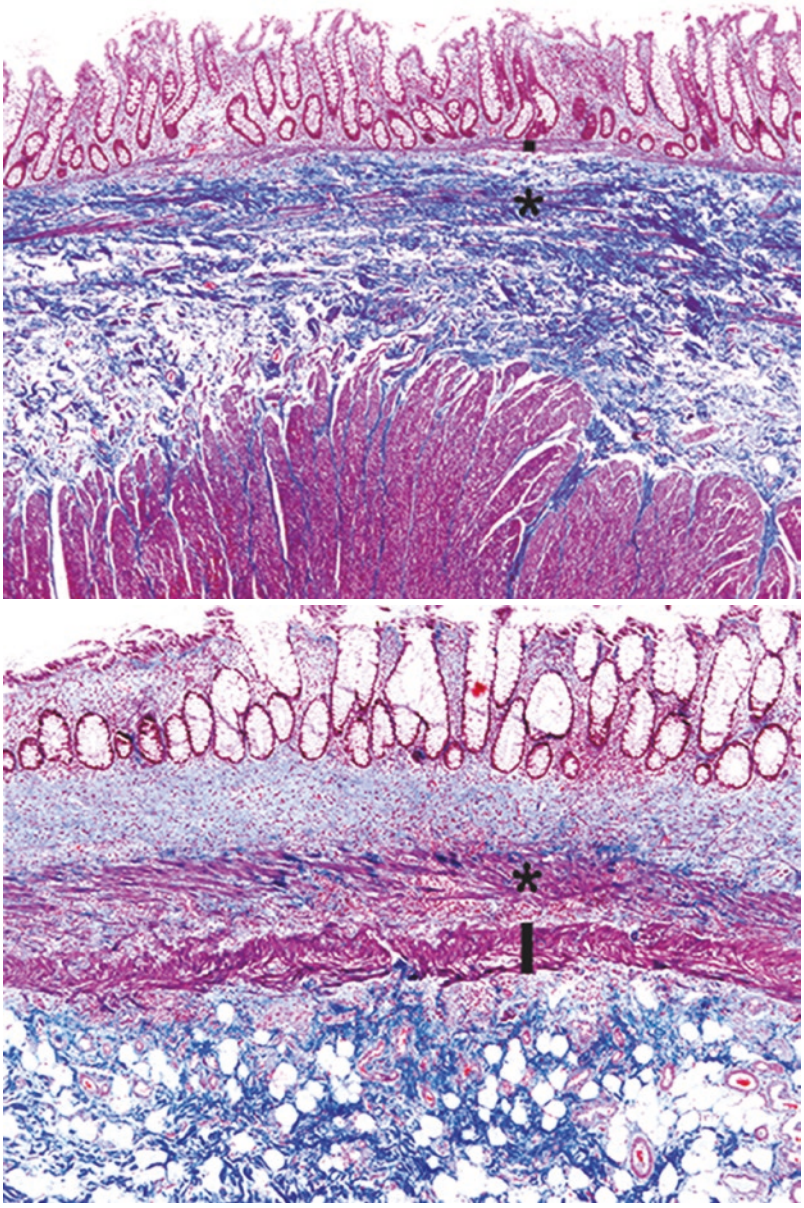

Fig. 11.7 Muscularis mucosae alterations in ulcerative colitis. In these two cases of ulcerative colitis with mucosal healing, contrast the thin and nearly intact muscularis mucosae with splayed muscularis hyperplasia within the superficial submucosa (Top), with the thickened muscularis mucosae with duplication on the mucosal aspect (Bottom). [black bar demarcates the original two layers of muscularis mucosae, black star indicates the alteration] (Masson Trichrome, original magnification 4×)

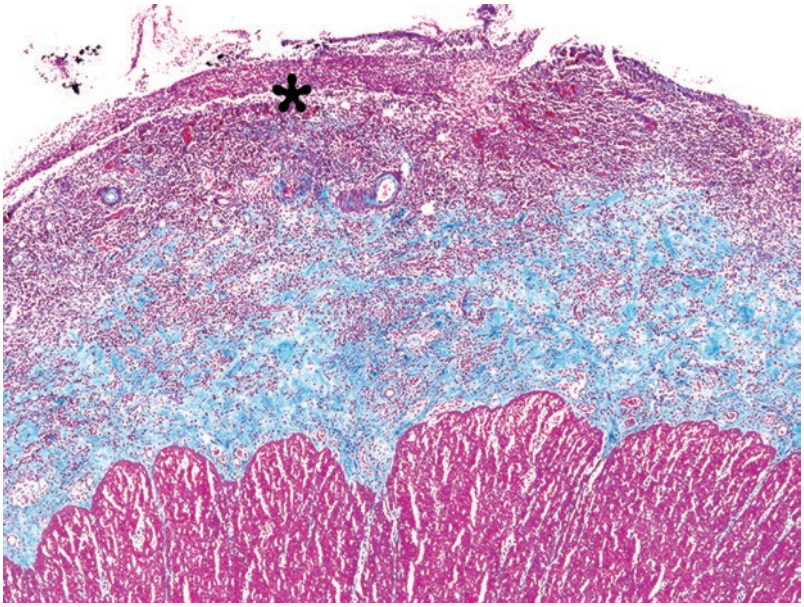

Fig. 11.8 Submucosal fibrosis in ulcerative colitis. Extensive ulceration (black star) is associated with fibrosis (blue) as part of the wound healing process (Masson Trichrome, original magnification 4×)

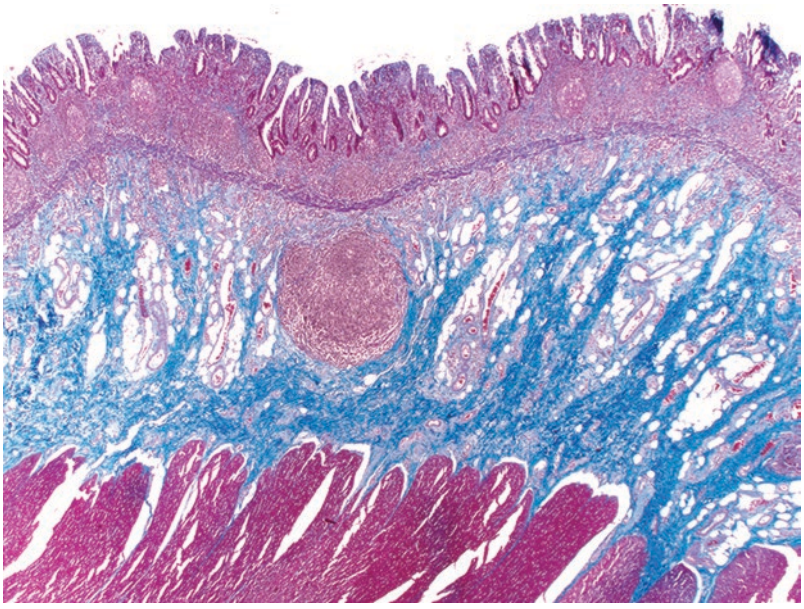

Fig. 11.9 Submucosal fibrosis in ulcerative colitis. Fibrous bands in the submucosa of non-ulcerated areas of ulcerative colitis typically form adjacent to the muscularis mucosae and muscularis propria and are otherwise perpendicular to the luminal flow (Masson Trichrome, original magnification 4×)

Table 11.1 Criteria for histologic fibrosis score of intestine

	Score	Description
Fibrosis	0	No increased collagen deposition
	1	Increased collagen deposition in submucosa
	2	Increased collagen deposition in submucosa and mucosa
	3	Increased collagen deposition in muscularis mucosa, submucosa, and mucosa; thickening, disorganization of the muscularis mucosa
	4	Increased collagen deposition in muscularis propria, muscularis mucosa, submucosa, and mucosa
	5	Increased collagen deposition throughout all layers including serosa
Percent involvement	1	0–25% of section
	2	25–50% of section
	3	50–75% of section
	4	75–100% of section

Note: used in rat model; based on Masson trichrome and Sirius Red stained sections
Reprinted from Theiss et al. [24], with permission

Table 11.2 Criteria for histologic fibrosis score

Score	Description
0	No fibrosis
1	Mild fibrosis (focal mucosal/submucosal collagen deposition without architectural distortion)
2	Moderate fibrosis (significant mucosal/submucosal collagen deposition with modest distortion of mucosal/submucosal architecture but without obscuring of the mucosal/submucosal border)
3	Severe fibrosis (extensive mucosal/submucosal collagen deposition with marked architectural distortion obscuring the mucosal/submucosal border)

Note: used in mouse model; based on Masson trichrome stained sections
Adapted from Higgins et al. [25], with permission

deposition (score 4), and finally all layers including serosa (score 5). Interestingly, there is also consideration of the percent of the section involved by fibrosis (Table 11.1), an important consideration given the segmental, and microscopically patchy, nature of Crohn's disease. The more recent mouse scoring system by Higgins et al. (Table 11.2) utilizes a progressive score considering only fibrosis (collagen deposition) affecting the mucosa and submucosa on Masson trichrome stained sections, with four tiers, from no fibrosis (score 0) to severe fibrosis (score 3). This scoring system is much less detailed than the earlier one, and has also been used on human tissue comparing fibrosis on pathology to ex vivo ultrasound evaluation in patients with inflammatory bowel disease [29].

Histologic scoring of fibrosis in studies comparing to radiographic findings are also somewhat less detailed, and do not include the percent section involvement, presumably due to examining only strictured segments. These studies use human tissue with Hematoxylin & Eosin stained sections. Adler et al. [26] separates fibrosis grade from muscle thickness, specifically thickening of the muscularis propria (Table 11.3). Unlike the earlier rodent scoring systems described above, this scoring

Table 11.3 Histologic evaluation scoring

Fibrosis grades	
0	No fibrosis
1	Minimal fibrosis in submucosa or subserosa
2	Increased submucosal fibrosis, septa into muscularis propria
3	Septa through muscularis propria, increase in subserosal collagen
4	Significant transmural scar, marked subserosal collagen
Muscle	
0	Normal thickness
1	Increased thickness of muscularis propria layer

Note: used in human tissue; based on H&E stained sections
Adapted from Adler et al. [26], with permission

Table 11.4 Scoring system for inflammatory and fibrostenotic features of Crohn's disease lesions

Fibrostenosis (score)	Pathology
None (0)	No or minimal fibrosis limited to submucosa (<25% thickness)
Mild/moderate (1)	Mild stricture (>15 mm) with nondilated lumen Submucosal fibrosis and muscular hyperplasia >25% with preserved layers
Severe (2)	Massive transmural fibrosis; effacement of normal layers; severe stricture

Note: used in human tissue; based on H&E stained sections
Adapted from Chiorean et al. [28], with permission

does not recognize mucosal fibrosis, or muscularis mucosae fibrosis or hyperplasia. Fibrosis is scored progressively, but considers submucosa, muscularis propria, and subserosa in each grade (Table 11.3). Chiorean et al. [28] takes a simplified approach with fewer score options in a three-tiered system (Table 11.4). Focus is again on submucosal fibrosis and on muscularis hyperplasia, though it is not specified whether this is referring to muscularis mucosae or muscularis propria.

Scoring schema from studies looking at resection specimen margins are probably the most generally applicable, as they consider pathologic features of Crohn's disease generally, not just findings from a stricture. Fibrosis of muscularis mucosae and submucosa are the focus, without specific mention of mucosa, muscularis propria, or subserosa, and a three-tiered progressive intensity grade was used [30, 31]. Interestingly, Maconi et al. [27] applied this scoring system, slightly modified to include four-tiers, to a group of Crohn's patients resected for ileal stenosis (stricture), where representative sections along the entire resected segment length were analyzed, and correlated the histologic findings with ultrasound echo patterns.

Neither the mouse nor the human scoring systems described here have been validated. As fibrosis in Crohn's disease is not generally thought to progress from

lumen to serosa, a scoring system that accounts for all layers of the bowel wall simultaneously is preferred. A similar scoring system should be applicable to both mice and human studies. The ideal Crohn's fibrosis scoring system would account for changes in the muscularis mucosae, including both hyperplasia and fibrosis, submucosal fibrosis and muscularization, muscularis propria hyperplasia and fibrosis, and subserosal fibrosis. A four-tiered scoring system (e.g. none, mild, moderate, severe) applied to each site (i.e. mucosa, muscularis mucosae, submucosa, muscularis propria, and subserosa) would be ideal, at least in early studies until clinical correlation could be established, and then perhaps the score could be contracted into three tiers. As Crohn's disease is patchy, multiple sections per specimen should be assessed, and a unified "per specimen" score could also be generated. Again, clinical correlation studies would be needed to best understand whether the unified score would reflect the highest overall score, or a combination of the scores from each section examined.

As the recognition and understanding of fibrosis in ulcerative colitis lags behind that of Crohn's disease, so does the concept of fibrosis scoring schema for ulcerative colitis. Measurement of the bowel wall layers has revealed muscularis mucosae thickening in ulcerative colitis [22], as well as increased fibronectin and collagen I in the mucosa, increased collagen I in the muscularis mucosae, and increased collagen I and III in the muscularis propria [32].

We developed a histologic fibrosis burden score for ulcerative colitis with a three-tiered approach focusing on the percent of submucosal fibrosis, and found moderate interobserver agreement on Hematoxylin & Eosin stained sections, and significant correlation when compared to Masson trichrome stain and Sirius red stain [22]. Bowel wall layer measurements revealed thickening of the muscularis mucosae, and this correlated with the presence of chronic mucosal injury [22].

11.4 Conclusion

In summary, fibrosis is a defining histopathologic feature of Crohn's disease. A variety of histologic scoring systems have been developed to evaluate fibrosis of inflammatory bowel disease, with a focus on Crohn's disease. In ulcerative colitis, recognition of histologic fibrosis has not been part of the diagnostic cadre, but has certainly been recognized and described in the literature. More studies evaluating the clinicopathologic correlation of fibrosis in ulcerative colitis are needed. Current histologic scoring systems reveal a variety of definitions of fibrosis and are incongruent as to the importance of fibrosis and mesenchymal cell hyperplasia in each of the bowel wall layers. Perhaps a unified histologic fibrosis scoring system, which can account for Crohn's disease, ulcerative colitis, strictured and non-strictured areas, and which incorporates the current understanding of the pathophysiology of fibrosis in inflammatory bowel disease, would be needed before widespread use could be established for clinical trials.

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