Journal of Crohn's and Colitis, 2025, **19(6)**, jjaf085
https://doi.org/10.1093/ecco-jcc/jjaf085
Advance access publication 20 May 2025
Original Article

Serum biomarkers of collagen remodeling are associated with intestinal fibrosis and differentiate stenotic from luminal Crohn's disease patients: a pre- and post-resection longitudinal study

Anja Poulsen[1,2,*], Marta Sorokina Alexdóttir[3], Lene Buhl Riis[2,4], Pernille Dige Ovesen[1,5], Julie Rasmussen[6], Mads Damsgaard Wewer[7,8], Viviane Lin[9], Ronja M.B. Lagström[9], Marwah Al-Sheikh[10], Emilie Dahl[1,5], Annedorte Ries[11], Martin Pehrsson[3], Thomai Tsapanou-Katranara[3], Peter-Martin Krarup[10], Ismail Gögenur[9], Florian Rieder[12,13,14], Johan Burisch[7,8], Joachim Høg Mortensen[3], Jakob Benedict Seidelin[1,2]

[1]Department of Digestive Diseases, Transplantation and General Surgery, Section for IBD, University Hospital of Copenhagen, Rigshospitalet, Copenhagen, Denmark
[2]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]Biomarkers and Research, Nordic Bioscience, Herlev, Denmark
[4]Department of Pathology, Copenhagen University Hospital, Herlev and Gentofte, Herlev, Denmark
[5]Department of Gastroenterology and Hepatology, Copenhagen University Hospital, Herlev and Gentofte, Herlev, Denmark
[6]Department of Medical Gastroenterology, Zealand University Hospital, Koege, Denmark
[7]Gastrounit – Medical Section, Copenhagen University Hospital – Amager and Hvidovre, Hvidovre, Denmark
[8]Copenhagen Center for Inflammatory Bowel Disease in Children, Adolescents and Adults, Copenhagen University Hospital – Amager and Hvidovre, Hvidovre, Denmark
[9]Center for Surgical Science, Zealand University Hospital, Koege, Denmark
[10]Digestive Disease Center, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark
[11]Department of Pathology, Copenhagen University Hospital – Amager and Hvidovre, Hvidovre, Denmark
[12]Program for Global Translational Inflammatory Bowel Disease, Cleveland Clinic, Cleveland, OH, United States
[13]Department of Inflammation and Immunity, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, United States
[14]Department of Gastroenterology, Hepatology and Nutrition, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH, United States

[*]Corresponding author: Anja Poulsen, Department of Digestive Diseases, Transplantation and General Surgery, Section for IBD, University Hospital of Copenhagen, Rigshospitalet, Inge Lehmanns Vej 5, entrance 3, 12th and 16th Floors2100 Copenhagen Ø, Denmark (anja.poulsen@regionh.dk).

Abstract

Background and Aims: Crohn's disease (CD) is characterized by progressive intestinal transmural damage, including fibrosis and strictures, which impair quality of life and require surgical intervention. No anti-stricture therapies are available, and no accurate biomarkers have been validated allowing prediction of strictures. Collagen fragments synthesis and remodeling show potential as markers of transmural disease activity. This study aimed to evaluate serum collagen markers for their accuracy in differentiating between stenosing and luminal CD and assessing their correlation with histopathology.

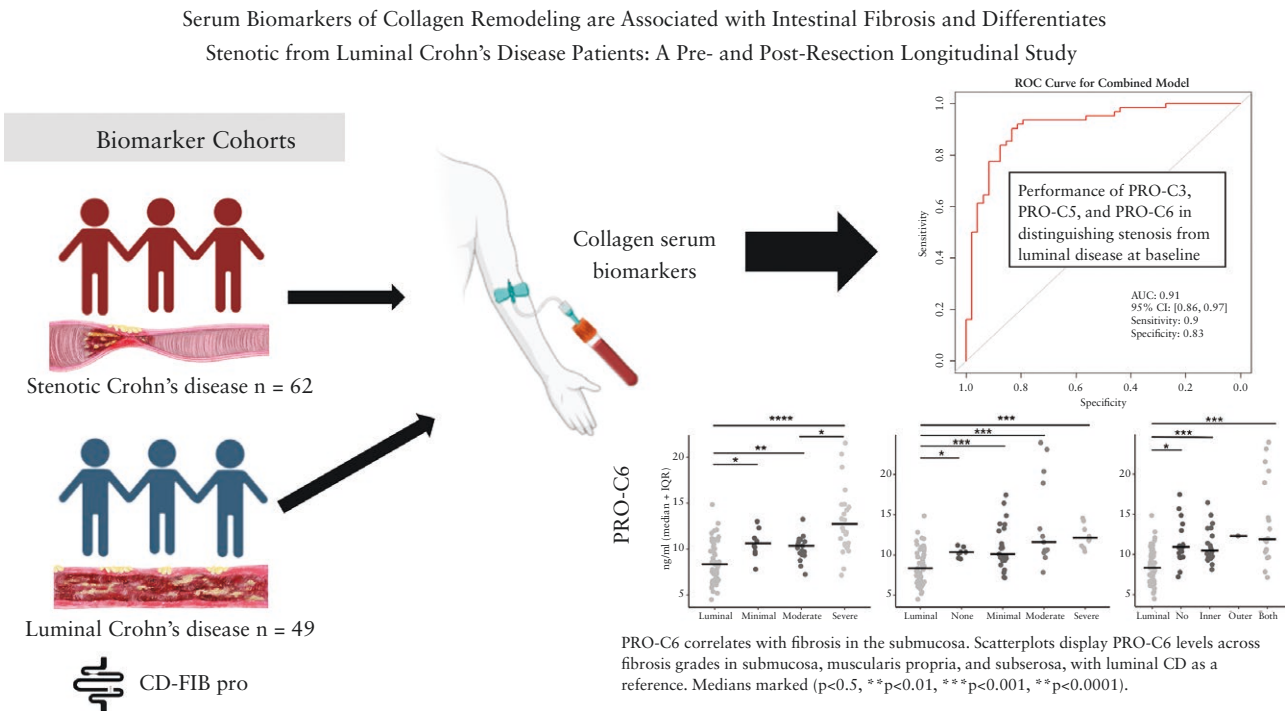
Methods: Sixty-two patients undergoing resection for stricturing CD and 49 with luminal CD were prospectively included. Extracellular matrix (ECM) markers were quantified using ELISA, and histological assessments of fibrosis and inflammation were performed on full-thickness tissue samples. Clinical outcomes, biomarkers, and histology were analyzed over a 12-month follow-up.

Results: Extracellular matrix markers, including PRO-C6, PRO-C3, PRO-C5, C4M, and PRO-C4, distinguished stenosing from luminal CD with and the combination of PRO-C6, PRO-C3, and PRO-C5 achieved the highest discriminative power of (AUC 0.91). Significant changes in levels of the collagen biomarker were observed post-resection. Histological analysis revealed extensive intestinal fibrosis in the submucosa of the stenotic segments, which correlated with PRO-C6 levels. C4M and PRO-C4 positively correlated with neutrophils in the lamina propria. CTX-III correlated negatively with the D'Haens score and neutrophils and mononuclear cells in the lamina propria and in the epithelium.

Conclusions: Collagen markers distinguished stenosing from luminal CD, and they correlated with histological fibrosis and chronic inflammation, promising for understanding ECM remodeling. This study highlights the need for extended follow-up to assess long-term stenosis-related outcomes.

Key words: Crohn's disease; collagen; biomarkers

Graphical abstract



PRO-C6 correlates with fibrosis in the submucosa. Scatterplots display PRO-C6 levels across fibrosis grades in submucosa, muscularis propria, and subserosa, with luminal CD as a reference. Medians marked (p<0.5, **p<0.01, ***p<0.001, **p<0.0001).

1. Introduction

Crohn's disease (CD) is an inflammatory bowel disease (IBD) distinguished by its segmental distribution and transmural involvement, potentially affecting any area of the gastrointestinal tract. Over time, CD can lead to progressive chronic damage in the intestines, with impaired tissue remodeling and fibrosis. Such complications frequently result in strictures, bowel obstruction, and subsequent fistulas.[1] These complications contribute to significant morbidity, often requiring repeated hospitalizations, surgical interventions, and carry a long-term risk of intestinal failure, stoma formation, and reduced quality of life.[2–6]

Fibrosis has traditionally been viewed as a result of sustained inflammation, causing continuous tissue injury and excessive buildup of extracellular matrix (ECM). Although there are many anti-inflammatory therapies available for CD, strictures continue to be a common complication, and there are currently no treatments that can effectively prevent or reverse fibrostenotic conditions.[2,3] The ECM is found in all tissues and is composed of the basement membrane as well as the interstitial matrix (IM). Basement membrane primarily consists of laminin and collagen types IV and VI, supporting epithelial and endothelial cells. In contrast, IM is rich in collagen types I, III, and V that provide structural support to the lamina propria and submucosa.[7–9] Differences in ECM protein expression and matrix metalloproteinase (MMP) levels, in part driven by mucosal inflammation, regulate homeostasis and the tissue's response to injury.[10] Disruptions in the balance between MMPs and their inhibitors (tissue inhibitor of metalloproteinases [TIMPs]) result in ECM breakdown and persistent scar formation.[11] Disease-driven excessive collagen cross-linking enhances fibroblast activity but can lead to pathological fibrosis.[12] Continuous physiological remodeling of the ECM not only maintains tissue integrity but is also essential in regulating processes of damage and repair. During remodeling, structural collagen molecules are released into the bloodstream as ECM collagen fragments, which may serve as biomarkers reflecting the extent of transmural remodeling activity within the intestinal wall.[7,9] Despite growing interest in ECM remodeling, few well-characterized study cohorts have been used to systematically evaluate a broad panel of collagen biomarkers reflecting distinct phases and compartments of ECM turnover. The biomarkers investigated in this study represent a comprehensive assessment of ECM remodeling activity, capturing both formation and degradation processes across different layers of the intestinal wall.[13] However, earlier investigations comparing stenotic and luminal disease have indicated that markers such as C1M, C3M, C4M, and PRO-C5 may help differentiate between these phenotypes.[14–16] PRO-C3 is also a well-established fibrosis marker in liver disease, although it has not yet been explored as a fibrosis marker in IBD. Endoscopy is only able to visualize the mucosal surface but is limited by its inability to biopsy or examine deeper layers beyond the mucosa, not reaching the submucosa where most ECM processes occur. In contrast, imaging methods such as computed tomography (CT), magnetic resonance enterography (MRE), and intestinal ultrasound allow full-thickness assessment but still face challenges in distinguishing fibrosis from inflammation.[17,18] Full-thickness tissue blocks from surgical specimens are considered the gold standard for grading fibrosis; however, standardized definitions for histological descriptions remain lacking.[19,20]

The development of precise biomarkers for inflammation, fibrosis, and stricture prediction could greatly enhance our understanding of potential anti-fibrotic targets and improve patient management through tailored therapies informed by accurate risk assessments. At present, there are no clinical biomarkers available for detecting intestinal fibrosis.[21] Extra cellular matrix fragments hold potential as markers, as they may offer a comprehensive view of transmural remodeling

activity within the intestinal wall. The aim of this study was to evaluate collagen markers related to ECM formation and degradation for their ability to distinguish between stenosing and non-stenosing CD, predict postoperative disease recurrence, and assess their correlation with the histological severity of fibrosis and inflammation.

2. Materials and methods

2.1. Study population

All patients were required to have a confirmed CD diagnosis according to the ECCO-ESGAR Guideline for Diagnostic Assessment in IBD and were divided into 2 groups described below.[22]

2.2. Patients with CD undergoing resection due to fibrostenotic disease

The criteria of inclusion were patients with stenotic CD that were scheduled for intestinal resection due to fibrostenotic complications, as defined by the CONSTRICT criteria for small bowel disease (lumen narrowing, bowel wall thickness, and/or pre-stenotic dilation) and non-passable stenosis at endoscopy for colonic strictures.[23] All patients underwent MRE before resection to assess the subtype and extent of the disease. Acutely admitted patients had an abdominal CT scan prior to resection. These scans were used to subtype CD patients' disease as having either stricturing (B2) or non-stricturing, non-penetrating disease (B1) according to the Montreal classification. Patients were excluded if there were areas of active disease not resected, patients were breastfeeding or pregnant, had ongoing cancer, inflammatory or fibrotic disease in joints, skin, pancreas, thyroid, lungs, or liver, or significant mental diagnosis hindering consent.

Prior to resection, the patients delivered blood and stool samples for analysis. Full-thickness intestinal tissue blocks from the resected specimen were collected from the stenotic area, as well as from the oral and anal margin to quantify the inflammatory and fibrotic burden. Study visits occurred the day after resection (POD), and at 1, 3, 6, and 12 months post-resection. Colonoscopy with biopsies from the anastomosis was performed within 1 year of follow-up and MRE at 12 months after surgery.

2.3. Patients with luminal CD

Patients were recruited just prior to treatment adjustment for their active disease (simple endoscopic score for Crohn's disease [SES-CD] ≥3 and/or inflammation at diagnostic imaging) and received conventional treatment as prescribed by the treating physician. Conventional treatment included optimization of existing therapies or initiation of new treatments deemed appropriate based on clinical judgment. Criteria of inclusion in the luminal group were non-stricturing and non-penetrating active CD according to the Montreal classification B1 demonstrated with endoscopies and diagnostic imaging (MRE) at inclusion.[24] Patients were excluded if they were pregnant or breastfeeding, ongoing cancer, use of nonsteroidal anti-inflammatory drug, activity in inflammatory or fibrotic disease in joints, skin, pancreas, thyroid, lungs, or liver, or significant mental diagnosis hindering informed consent.

At inclusion (prior to treatment adjustment), patients underwent ileocolonoscopy with biopsies, MRE along with collection of regular and biobanked blood samples and fecal calprotectin.

2.4. Data collection

Patient information was collected from medical records from the diagnosis to the 12-month follow-up period in the stenotic group and at baseline only in the luminal group. This included demographic details such as age, height, weight, sex, smoking status, duration of disease, symptoms, co-morbidities, past and current IBD-related medications, laboratory findings, and results from diagnostic imaging and endoscopy. The data were managed through the REDCap online electronic database (Research Electronic Data Capture, The Capital Region of Denmark). Biological samples (blood components and biopsies) were preserved in a research biobank.

2.5. Endoscopic and clinical definitions

All colonoscopies were scored using the SES-CD. We defined SES-CD remission (0-2), mild (3-6), moderate (7-15), and severe disease (≥16).[25] Colonoscopies performed after resection were scored using the Rutgeerts score, with ≥i2b indicating disease activity at the anastomosis.[26] The markers referred to in the result section as "routine markers" in this context included hemoglobin, platelets, albumin, C-reactive protein (CRP), and fecal calprotectin.

2.6. Biomarkers and assay measurements

Blood was collected in 9 mL gold-top SST tubes, gently inverted 5 times, and left to clot at room temperature for 30–60 minutes. Samples were centrifuged at 2000 × *g* for 15 minutes, and serum was aliquoted into 3 cryotubes and frozen at –80 °C within 30 minutes. We examined the following neo-epitope markers of the ECM: collagen I (C1M), collagen III (C3M, PRO-C3, CTX-III), collagen IV (C4M, C4G, PRO-C4), collagen V (PRO-C5), collagen VI (C6M, PRO-C6, C6Ma3), collagen VII (PRO-C7), collagen XI (PRO-C11), collagen XVI (PRO-C16), collagen XVII (PRO-C17), and collagen XXII (PRO-C22) (Table 1). They were quantified using competitive ELISA, with each sample analyzed in duplicate. Serum samples were incubated on streptavidin-coated 96-well microplates with specific biotinylated antigens, followed by washing and exposure to a horseradish peroxidase-conjugated antibody. Detection was performed via colorimetric or chemiluminescence methods, measuring absorbances with a VersaMAX ELISA microplate reader or a Fluroskan fluorescence plate reader. Detailed assay conditions and standard curves were established as per the protocol outlined in the technical papers (Table S1).

2.7. Histological description

Full-thickness tissue blocks from the stenosis as well as oral and anal margins were collected from the resected specimens and embedded in paraffin. Sections from each area were cut and stained with hematoxylin and eosin (H&E), which was considered appropriate for the histopathological evaluation in this study.[19] All sections were blindly centrally reviewed by the same IBD pathologist.

The description of fibrosis in the full-wall tissue slides was categorized as amount of fibrosis: none (grade 0), minimal (grade 1), moderate (grade 2), and severe (grade 3) for each layer corresponding to the submucosa, subserosa, muscularis propria, and "muscularization" of the submucosa on a binary scale: yes or no, with the latter graded as the most severe measure of fibrosis (Figure 1). We also recorded the absence or presence

Table 1. A description of the ECM neo-epitope specific biomarkers measured in the study at hand, their implications, and references to their respective technical paper.

Biomarker	Description	Implication	References
C1M	MMP-2/9/13-degraded type I collagen α1-chain	Interstitial matrix degradation	[27]
C3M	MMP-9-degraded type III collagen	Interstitial matrix degradation	[28]
PRO-C3	N-terminal pro-peptide of type III collagen	Formation of type III collagen	[29]
C4M	MMP-2/9/12-degraded type IV collagen α1-chain	Basement membrane degradation	[30]
C4G	GrzB-degraded type IV collagen α2-chain	T-cell activity and basement membrane degradation	[31]
PRO-C4	7s domain of type IV collagen (internal epitope)	Basement membrane turnover	[32]
PRO-C5	C-terminal pro-peptide of type V collagen α2-chain	Interstitial matrix formation, collagen fibril formation	[33]
C6M	MMP-2 degraded type VI collagen α1-chain	Interstitial matrix degradation	[34]
PRO-C6	Released C-terminal C5 domain of type VI collagen α3-chain	Interstitial matrix formation	[35]
C6Ma3	MMP-2/9-degraded type VI collagen α3-chain	Interstitial matrix degradation	[36]
PRO-C7	NC2 pro-peptide of type VII collagen α1-chain	Formation of type VII collagen	Unpublished
PRO-C11	C-terminal pro-peptide of type XI collagen α1-chain	Formation of type XI collagen	[37]
PRO-C16	C-terminal of type XVI collagen α1-chain (internal epitope)	Type XVI collagen turnover	[38]
PRO-C17	Ectodomain of type XVII collagen α1-chain	Type XVII collagen turnover	[39]
PRO-C22	A fragment of C-terminal type XXII collagen	Turnover of type XXII collagen	[40]
CTX-III	MMP-9 and -13 released C-terminal telopeptide of type II collagen	The release of cross-linked type III collagen, fibrolysis	[41]

Abbreviations: ECM, extracellular matrix; MMP, metalloproteinase.

of granulomas and transmural inflammation. Additionally, D'Haens histological scoring was performed for grading the histological inflammatory disease activity.[42] The D'Haens score is composed of a combined assessment for epithelial damage, architectural damage, and mononuclear infiltration in the lamina propria, categorized as a score from 0 to 2, ranging from normal to severe. Neutrophils in the lamina propria were scored from 0 to 2, from normal to severe, and neutrophils in the epithelium were scored from 0-3 (no neutrophils, in surface epithelium, cryptitis, crypt abscesses). We also recorded the presence or absence of ulcers or erosions.[19,20,42–44] In cases with severe ulcerations and absence of mucosa, segmental scoring according to the D'Haens was not possible. As a result, these segments were excluded from the total score calculation.

2.8. Statistical analyses

Baseline characteristics were evaluated with nonparametric statistical methods. Continuous variables are presented as median values with IQRs, while categorical variables are shown as counts (n) with corresponding percentages. The distribution of continuous variables was assessed by visually inspecting Q-Q plots and histograms. The Wilcoxon Rank Signed Sum test was used to compare biomarkers in stenotic CD before and after resection and with the luminal cohort. Additionally, the test was applied to compare biomarkers between patients with and without perianal disease, and between those undergoing open resection, within the resected group. The ability to predict stenosing vs luminal disease as well as disease recurrence within 1 year after resection was evaluated using receiver operating characteristic analysis, using the area under the curve (AUC). The combination of biomarkers was analyzed using a logistic regression model to evaluate their ability to differentiate between the stenosis and luminal phenotypes at baseline. A logistic regression model was used to assess the association between smoking status, gender, and age at inclusion with recurrence risk, reported as odds ratios (ORs) with 95% CIs.

Sample size calculations were conducted using G*Power (Version 3.1.9.2) to detect a 20% difference in serum levels of individual collagen biomarkers between patients with stenotic and luminal CD at baseline, assuming a standard deviation corresponding to a large effect size (Cohen's d = 0.8) and a power of 95%. This resulted in an estimated minimum sample size of 42 subjects per group; however, we opted to include 50 in each group. The stenotic group initially included 63 subjects to ensure that 50 participants would complete the 4-year follow-up, with early dropouts replaced within the first year, leading to a higher initial count. Statistical analyses were conducted in R for Windows (version 4.3.2), supported by RStudio (version 2024.04.2). Significance was set at $P < .05$.

2.9. Ethical considerations

Approval was secured from the Regional Scientific Committee (H-20065801) and the Danish Patient Safety Authority (P2020-609). Additionally, the study received authorization from the local institutional review board for its local implementation, along with written consent from the patients.

3. Results

3.1. Cohort characteristics

Sixty-two patients undergoing resection for stricturing CD and 49 with luminal CD and flaring were prospectively

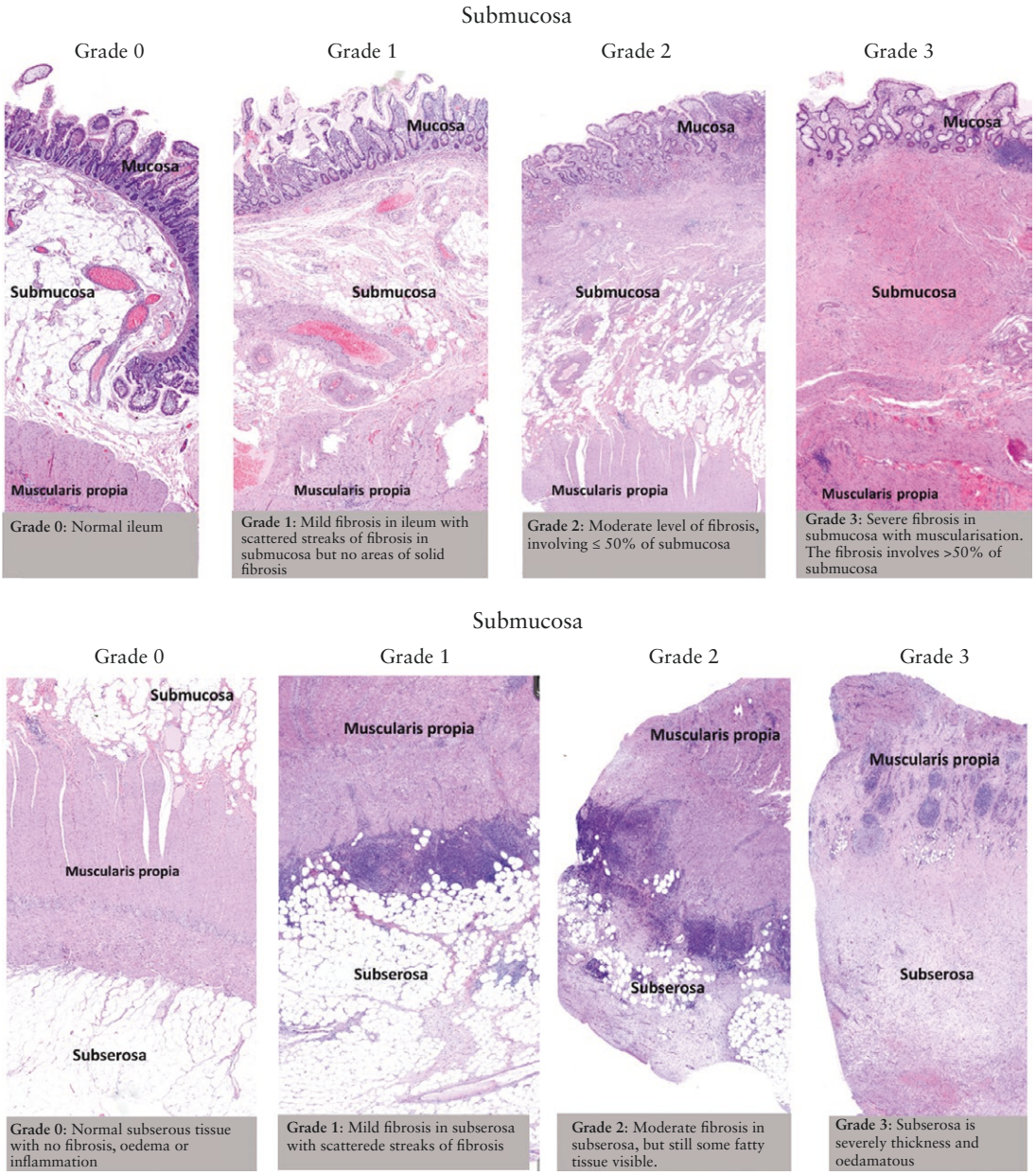


Figure 1. The fibrosis in full-wall tissue blocks was categorized by amount as follows: none (grade 0), minimal (grade 1), moderate (grade 2), and severe (grade 3). This classification was applied separately to each layer, shown here for the submucosa and subserosa.

included. In the group of patients with stricturing disease, the median age at inclusion was 42 years (IQR: 31-55), and 31 (50%) were men. The distribution of disease location was as follows: terminal ileum, L1: 33 (53%), colon, L2: 7 (11%), ileocolonic L3: 22 (36%). A total of 19 patients (30%) had undergone prior resection. In the stenotic group, 54 (87%) patients underwent a colonoscopy within the first year after resection, with a median time from resection to endoscopy at 293 days (IQR: 223-354). A total of 20 patients (32%) had a Rutgeerts score of ≥i2b, consistent with disease recurrence, of which 4 patients (6%) had a score of i3, and 2 patients (3%) had a score of i4. For the luminal group, the median age at inclusion was 30 (25-41), and 31 (63%) were men. The distribution of disease location was as follows L1: 10 patients (20%), L2: 22 (45%), L3: 17 (35%), and L4: none. The endoscopic SES-CD at inclusion revealed mild disease in 19 patients (39%), moderate in 20 (41%) severe in 3 (6%), and 7 patients (14%) had a normal colonoscopy but exhibited signs of disease beyond endoscopic reach as detected by diagnostic imaging (Table 2).

3.2. Baseline demographics and biomarker correlations in stenotic CD

When examining the baseline demographics of the stenotic CD population in relation to the ECM markers PRO-C4 and PRO-C6 correlated with disease duration ($\rho = -0.282$, $P = .026$ and $\rho = -0.296$, $P = .017$, respectively). C6MA3 correlated with the length of the resected specimen ($\rho = 0.294$, $P = .020$). None of the markers correlated with BMI. PRO-C6 and PRO-C22 correlated negatively with albumin ($\rho = -0.306$, $P = .017$, $\rho = -0.363$, $P = .004$, respectively) and PRO-C22 further correlated with CRP ($\rho = 0.316$, $P = .012$)

Table 2. Baseline characteristics for both the stenotic and luminal groups, and 12-month follow-up data for the stenotic group.

	Stenosis baseline	Stenosis 12 months	Luminal baseline
	n = 62 (100%)	*n* = 62 (100%)	*n* = 49 (100%)
Gender (male)	31 (50%)	-	31 (63%)
Age at diagnosis median (IQR)	29 (22-39)	-	25.5 (20.3-31.0)
Age at inclusion median (IQR)	42 (31-55)	-	30 (25.0-41.0)
Years with disease median (IQR)	7.2 (1.7-12.5)	-	2.7 (0.3-8.0)
BMI median (IQR)	24.9 (21.7-28.8)		23.3 (21.3-26.0)
Smoking			
- Active smoker	22 (35%)	20 (32%)	4 (8%)
- Former smoker	14 (23%)	16 (26%)	17 (35%)
- Never	26 (42%)	26 (42%)	28 (57%)
Familiar disposition	21 (34%)	-	15 (31%)
HBI median (IQR)	6 (3-9)	3 (2-6)	5 (2-8)
- Remission (< 5)	16 (26%)	25 (40%)	23 (47%)
- Mild (5-7)	13 (21%)	12 (19%)	13(27%)
- Moderate (8-16)	31 (50%)	9 (15%)	12 (24%)
- Severe < 16	2 (3%)	0	0
Diagnostic imaging	62 (100%)	49 (79%)	49 (100%)
Colonoscopy	-	53 (85%)	49 (100%)
SES-CD median (IQR)	-	1 (0-4)	6 (3-12)
- Remission (0-2)	-	32 (52%)	7 (14%)
- Mild (3-6)	-	16 (26%)	19 (39%)
- Moderate (7-15)	-	4 (6%)	20 (41%)
- Severe (≥16)	-	1 (<2%)	3 (6%)
Disease location			
- L1 (T. ileum)	33 (53%)	32 (52%)	10 (20%)
- L2 (colon)	7 (11%)	7 (11%)	22 (45%)
- L3 (Ileocolonic)	22 (36%)	23 (37%)	17 (35%)
- L4 (upper)	0	1 (<2%)	0
Disease behavior			
- B1 (luminal)	0	0	49 (100%)
- B2 (stenotic)	62 (100%)	62 (100%)	0
- B3 (penetrating (+ B2))	11 (18%)	11 (18%)	0
- +P (perianal)	8 (13%)	8 (13%)	8 (16%)
Prior resected	19 (31%)	62 (100%)	0
IBD medication			
- Systemic corticosteroids	5 (8%)	3 (5%)	9 (18%)
- Immunomodulators	6 (10%)	4 (6%)	13 (27%)
- Biologics	24 (39%)	36 (58%)	16 (33%)
-Anti-TNF	11 (18%)	21 (34%)	14 (29%)
- Vedolizumab	5 (8%)	6 (10%)	1 (2%)
- Ustekinumab	8 (13%)	9 (15%)	1 (2%)
- Small molecules	0	0	0
- No medication	30 (48%)	23 (37%)	14 (29%)

Abbreviation: IBD, inflammatory bowel disease; BMI, body mass index; HBI, Harvey-Bradshaw index; TNF, tumor necrosis factor.

(Table 3). None of the markers at baseline showed a significant difference between patients with and without perianal disease or Montreal disease location.

3.3. Comparing stenotic and luminal CD using ECM biomarkers

The concentrations of several ECM biomarkers differed significantly between the stenotic and luminal CD cohorts at baseline. The largest differences were observed for PRO-C3, PRO-C5, and PRO-C6, which were further evaluated in a combined model. The median concentration of the ECM markers at baseline differentiated the stenotic CD cohort from the luminal cohort (Figure 2) with elevated PRO-C3 (57.26 vs 43.40 ng/mL, $P < .001$), C4M (270.75 vs 245.00 ng/mL, $P = .004$), PRO-C4 (7295.80 vs 6592.00 ng/mL, $P < .001$), PRO-C5 (369.50 vs 280.00 ng/mL, $P < .001$), PRO-C6 (11.06 vs 8.35 ng/mL,

Table 3. In the stenotic cohort, Spearman's correlation was used to analyze the demographics, serological ECM markers, and routine blood markers.

	C3M		**PRO-C3**		**C4G**		**C4M**	
	Roh	***P*-value**	**Roh**	***P*-value**	**Roh**	***P*-value**	**Roh**	***P*-value**
Hemoglobin	0.039	.763	0.004	.973	−0.024	.852	0.072	.579
Platelets	***0.277***	***.031***	0.067	.607	0.014	.913	***0.274***	***.033***
Albumin	−−0.024	.855	−0.041	.753	−0.001	.996	−0.082	.528
CRP	0.061	.639	0.148	.249	−0.011	.931	0.089	.488
Fecal calprotectin	−0.216	.159	−0.085	.585	0.269	.077	−0.048	.759
Length of specimen	−0.023	.862	−0.121	.349	0.145	.262	−0.014	.914
BMI	−0.000	.998	−0.015	.909	−0.232	.069	−0.035	.785
Age at inclusion	0.147	.254	−0.139	.278	***−0.308***	***.015***	***0.251***	***.049***
Disease duration	−0.234	.067	−0.207	.106	−0.142	.269	−0.127	.324
	PRO-C4		**PRO-C5**		**C6M**		**PRO-C6**	
	Roh	*P*-value	Roh	*P*-value	Roh	*P*-value	Roh	*P*-value
Hemoglobin	0.012	.928	0.185	. 149	−0.041	.750	***−0.282***	***.027***
Platelets	0.134	.302	0.125	.336	0.163	.209	0.119	.359
Albumin	−0.103	.429	−0.080	.540	−0.232	.072	***−0.306***	***.017***
CRP	0.156	.228	0.092	.476	0.214	.094	0.104	.423
Fecal calprotectin	−0.197	.201	0.043	.782	−0.027	.864	0.229	.153
Length of specimen	0.006	.963	0.206	.108	0.025	.848	0.049	.707
BMI	0.055	.671	0.037	.773	−0.068	.598	−0.147	.252
Age at inclusion	0.167	.193	0.053	.685	0.145	.262	***−0.250***	***.049***
Disease duration	***−0.282***	***.026***	0.020	.878	−0.146	.257	***−0.296***	***.019***
	C6MA3		**PRO-C7**		**PRO-C11**		**PRO-C16**	
	Roh	*P*-value	Roh	*P*-value	Roh	*P*-value	Roh	*P*-value
Hemoglobin	0.116	.371	0.052	.687	0.035	.790	0.175	.174
Platelets	0.045	.729	−0.041	.755	0.089	.501	0.080	.539
Albumin	−0.143	.270	−0.181	.163	−0.193	.139	−0.059	.650
CRP	0.093	.471	−0.051	.610	0.085	.514	0.129	.317
Fecal calprotectin	−0.015	.924	−0.052	.739	0.131	.398	−0.102	.511
Length of specimen	***0.294***	***.020***	0.095	.464	0.019	.883	0.104	.419
BMI	0.006	.961	0.020	.876	−0.022	.869	0.029	.822
Age at inclusion	0.110	.393	0.210	.101	−0.014	.912	0.062	.630
Disease duration	0.011	.930	0.106	.411	−0.117	.367	−0.072	.580
	PRO-C17		**PRO-C22**		**CTX-III**			
	Roh	*P*–value	Roh	*P*–value	Roh	*P*–value		
Hemoglobin	−0.061	.635	−0.152	.238	0.228	.074		
Platelets	0.009	.946	0.192	.138	−0.029	.827		
Albumin	−0.145	.266	***−0.363***	***.004***	0.175	.178		
CRP	0.072	.579	***0.316***	***.012***	−0.004	.974		
Fecal calprotectin	−0.081	.599	0.086	.581	0.012	.938		
Length of specimen	−0.059	.649	0.109	.398	0.064	.623		
BMI	−0.054	.675	−0.141	.273	0.146	.257		
Age at inclusion	−0.159	.215	0.052	.688	0.046	.720		
Disease duration	−0.089	.493	−0.216	.092	−0.001	.994		

Significant findings ($P < .05$) are highlighted in bold.

P < *.001*), PRO-C16 (2676.00 vs 2960.00 ng/mL, *P* < *.001*), PRO-C17 (4.35 vs 3.05 ng/mL, *P* = *.008*), C3M/PRO-C3 (5.10 vs 13.81 ng/mL, *P* < *.001*), and C6M/PRO-C6 (88.92 vs 111.93 ng/mL, *P* < *.001*). Routine markers of inflammation showed overall more inflammatory activity in the stenotic group vs the luminal group: The stenotic cohort had a lower median hemoglobin (8.2 vs 8.5 mmol/L, *P* = *.026*), lower albumin (35 vs 38 g/L, *P* = *.003*), elevated CRP (5 vs 2 mm/L,

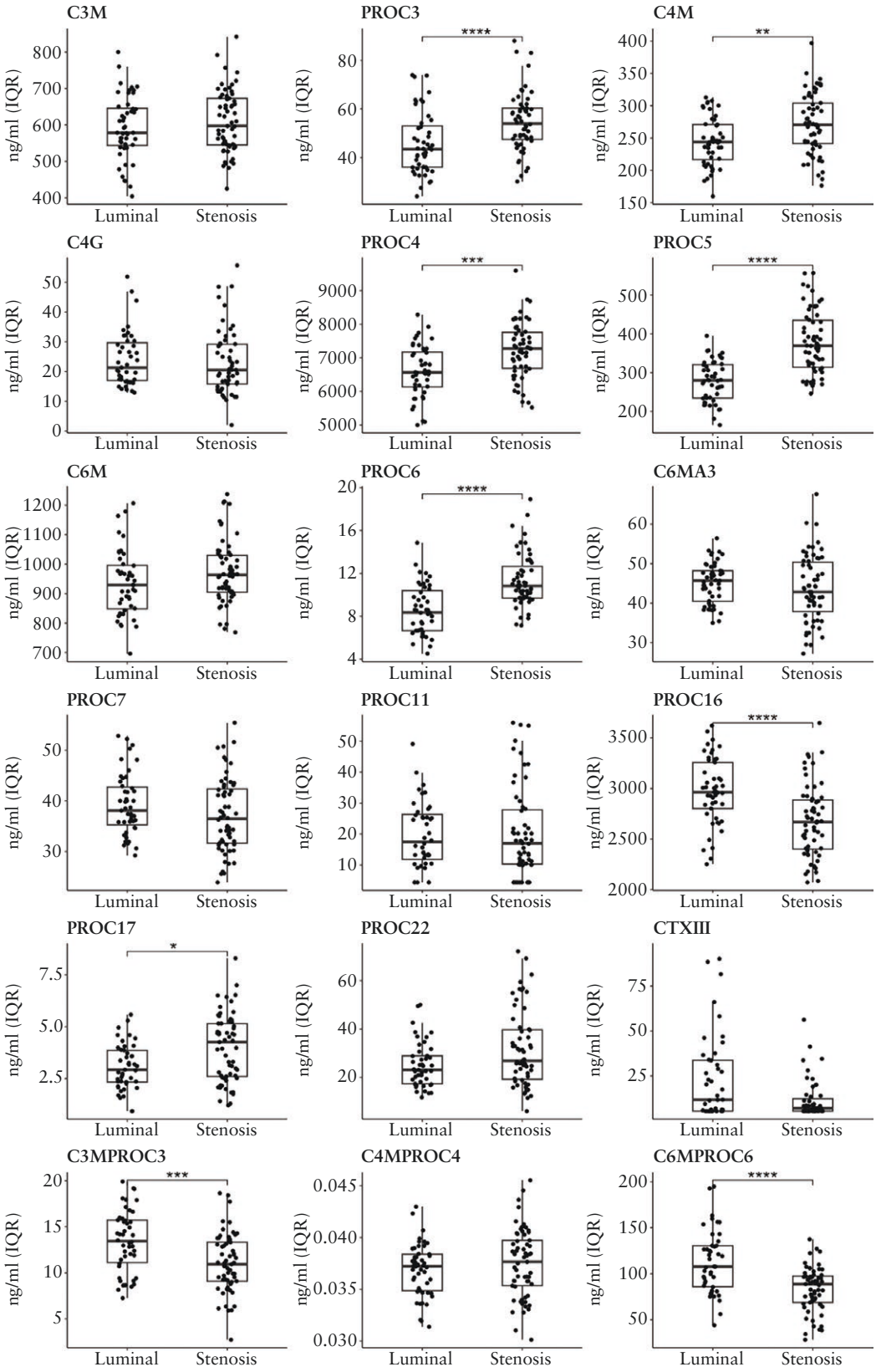


Figure 2. Median concentration with IQRs for the individual biomarkers for all baseline visits in the patient group with luminal CD compared to stenotic CD.* $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$.

$P < .001$). However, fecal calprotectin was higher in the luminal group (861 vs 286 mg/kg, $P = .006$) (Table S2). When we selected the 3 best-performing markers based on their AUC ability to distinguish stenosis from luminal disease at baseline (PRO-C3, PRO-C5, and PRO-C6) and performed a logistic regression, the combined model demonstrated a significant

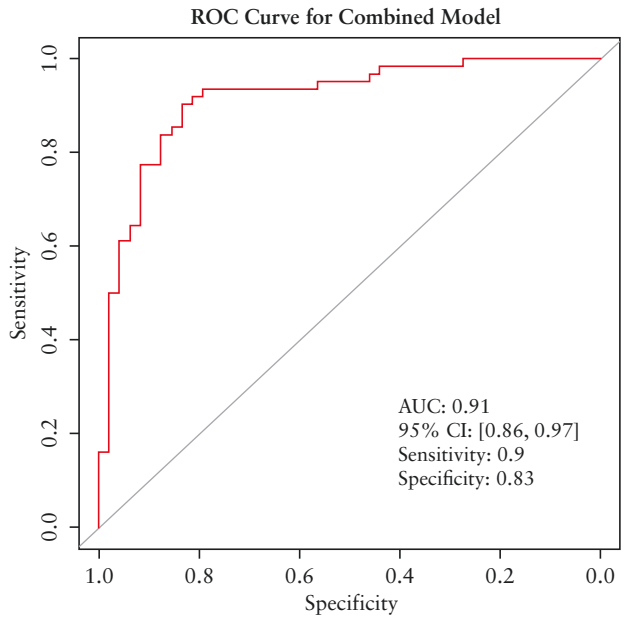


Figure 3. Receiver operating characteristic (ROC) curve for the combined logistic regression model using the 3 best-performing markers at baseline (PRO-C3, PRO-C5, and PRO-C6), selected based on their AUC ability to distinguish stenosis from luminal disease. AUC, area under the curve.

differentiation between stenosis and luminal disease with an AUC of 0.91 and a 95%CI, 0.86–0.97 (Figure 3).

In the stenotic cohort, using each patient as their own control, we observed a significant postoperative change, with several ECM markers showing isolated marked changes at POD compared to baseline. At POD, C3M decreased from a baseline median of 601.5 ng/mL (IQR: 545.5–675.3) to 396.5 ng/mL (IQR: 332.8–483.3), $P < .001$. Similarly, PRO-C3 levels dropped from 57.26 ng/mL (IQR: 47.65–75.69) pre-resection to 45.69 ng/mL (IQR: 35.63–56.57), $P < .001$. In contrast, C4G increased from 22.36 ng/mL (IQR: 16.45–34.16) to 27.14 ng/mL (IQR: 19.49–39.79), $P = .002$, while PRO-C4 declined from 7296 ng/mL (IQR: 6690–7880) to 5564 ng/mL (IQR: 4609–6396), $P < .001$, $P < .001$ at POD. PRO-C11 increased from a baseline of 17.53 ng/mL (IQR: 10.53–28.74) to 23.02 ng/mL (IQR: 17.83–35.02), $P < .001$ at POD. Among the routine clinical markers, albumin levels decreased significantly following resection, from 35 g/L (IQR: 8.5) to 30 g/L (IQR: 6.5), $P < .0001$ (Figure 4, Table S3).

When we looked at the ECM marker progression from pre-resection to 1-year post-resection in fibrostenotic patients, several markers exhibited sustained significant changes over time. Notably, C4M levels were significantly reduced from a baseline median of 270.75 ng/mL (IQR: 241.93–310.40) to a median of 240.60 ng/mL (IQR: 218.90–285.20) at 12 months ($P < .001$ across multiple time points). Similarly, PRO-C5 levels initially dropped significantly postoperatively ($P < .001$) and remained lower than baseline through 6 months ($P = .015$), though this difference was not sustained at 12 months ($P = .088$). C6M showed a persistent decrease over the entire follow-up period, from a pre-resection median of 966.00 ng/mL (IQR: 904.75–1039.25) to 891.00 ng/mL (IQR: 799.00–944.00) at 12 months ($P < .001$ at all-time points). C6MA3 levels dropped significantly postoperatively ($P < .001$) and remained below baseline through 6 months ($P = .002$); however, this difference was not sustained at 12 months ($P = .063$). Further, PRO-C7 exhibited significant reductions from baseline at several time points, with values decreasing postoperatively and remaining below pre-resection levels through 6 months ($P = .002$), though this effect disappeared by 12 months ($P = .429$). PRO-C16 levels increased progressively over the postoperative period, with a baseline median of 2676.00 ng/mL (IQR: 2407.25–2907.25) rising to 2934.00 ng/mL (IQR: 2691.00–3235.00) by 12 months, a change that remained significant ($P < .001$ at multiple time points). PRO-C22 consistently decreased from a pre-resection median of 29.01 ng/mL (IQR: 19.76–43.31) to 17.94 ng/mL (IQR: 13.37–23.29) at 12 months, with statistically significant differences observed at each postoperative time point ($P < .001$) (Figure 4). Sustained post-surgical changes in ECM dynamics could reflect decreased post-surgical fibrosis burden.

3.4. Characterization of histological features in resected specimens

Full-thickness tissue samples from the resected specimens revealed significantly increased fibrosis and inflammation at the stenotic site compared to the oral and anal margins. All resected specimens from patients displayed fibrosis at the stenotic region. Among these, 27 patients (44%) showed severe submucosal fibrosis, 23 (37%) moderate, 12 (19%) mild, and none were free from fibrotic changes. Fibrosis was identified at the oral resection margin in 20 patients (32%), with submucosal involvement in 15 (24%). At the anal margin, fibrotic changes were present in 13 patients (21%), including submucosal involvement in 12 (19%). The D'Haens histological score indicated a higher degree of inflammation in the stenotic area, with 61 patients (97%) showing a positive inflammation outcome (D'Haens ≥1) and a median score of 9 (IQR: 4–11). In contrast, active inflammation was observed at the oral margin in 9 patients (14%) and at the anal margin in 21 patients (33%) (Table 4).

3.5. Tracking fibrosis and inflammation: linking biomarkers with full-wall tissue blocks

PRO-C6 correlated with the degree of fibrosis in the submucosa ($\rho = 0.304$, $P = .017$), divided into fibrosis grade 1, 2, and 3 but not with fibrosis of the other intestinal wall layers. PRO-C6 was, however, significantly elevated for all fibrosis gradings compared to luminal CD patients (Figure 5). No other ECM markers correlated with the burden of intestinal wall fibrosis (Table 5). Routine inflammation markers correlated with D'Haens histological score for inflammation in the resected specimen: albumin and fecal calprotectin ($\rho = -0.473$, $\rho = 0.399$, both $P < .01$). Specifically looking at lamina propria neutrophil infiltration revealed a positive correlation with CRP and fecal calprotectin, whereas the severity markers albumin and hemoglobin correlated negatively with lamina propria neutrophils as well as submucosal fibrosis. The cross-linked type III collagen, as indicated by CTX-III, correlated overall with the D'Haens score ($\rho = -0.276$, $P = .048$), including subcomponents such as epithelial damage ($\rho = -0.285$, $P = .029$), mononuclear lamina propria infiltration ($\rho = -0.358$, $P = .008$), and epithelial neutrophils ($\rho = -0.335$, $P = .015$) (Table S4). PRO-C3, C4M, and PRO-C4 correlated negatively with mononuclear cells in

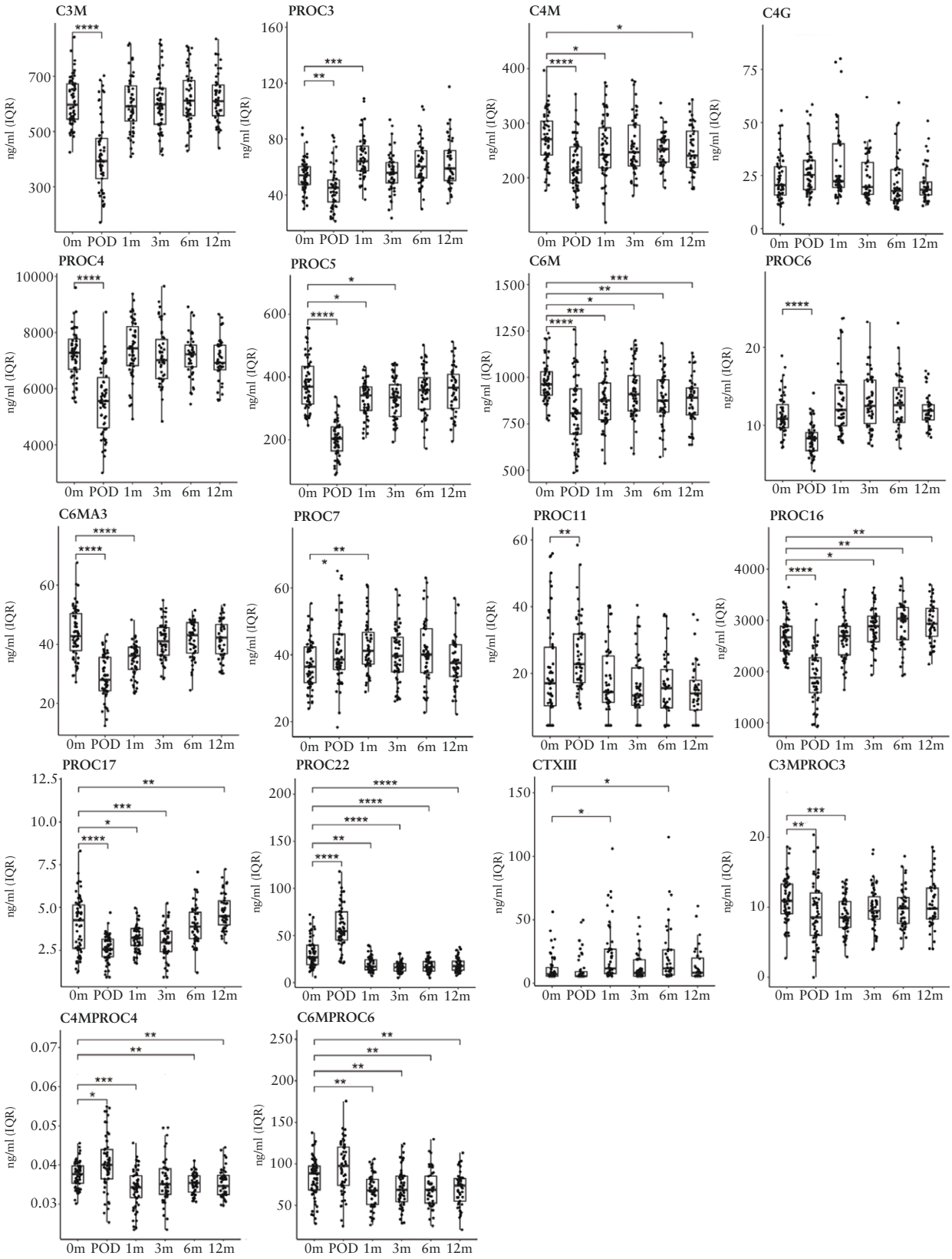


Figure 4. The course of ECM markers measured pre-resection, at POD, and at 1, 3, 6, and 12 months postoperatively. At each time point, the median value with the corresponding IQR is displayed. For each postoperative time point, a *P*-value (paired) was calculated to determine whether there is a significant difference compared to the pre-resection value. *P* *< .05, ** *P* < .01, *** *P* < .001, **** *P* < .0001. ECM, extracellular matrix; POD, day after resection.

Table 4. Histological scoring of the layers in the intestinal wall of the stenotic area, categorized by grading and expressed as number and percentage.

Stenoses ($n = 62$)	Grade 0	Grade 1	Grade 2	Grade 3	Yes	No	N/A
Fibrosis in submucosa	0	12 (19%)	23 (37%)	27 (44%)	-	-	0
Fibrosis in subserosa	11 (18%)	28 (45%)	12 (19%)	11 (18%)	-	-	0
Fibrosis in muscularis propria	21 (34%)	22 (35%)	1 (<2%)	18 (29%)	-	-	0
Muscularization of submucosa	-	-	-	-	32 (52%)	30 (48%)	0
Epithelial damage	9 (15%)	10 (16%)	40 (64%)	-	-	-	3 (5%)
Architectural damage	5 (8%)	17 (27%)	32 (52%)	-	-	-	8 (13%)
Mononuclear in lamina propria	6 (10%)	17 (27%)	31 (50%)	-	-	-	8 (13%)
Neutrophils in lamina propria	15 (24%)	20 (32%)	18 (29%)	0	-	-	9 (15%)
Neutrophils in the epithelium	11 (18%)	17 (27%)	14 (23%)	10 (16%)	-	-	10 (16%)
Ulcer/erosion	-	-	-	-	41 (66%)	21 (34%)	0
Granulomas	-	-	-	-	9 (15%)	53 (85%)	0
Transmural inflammation	-	-	-	-	41 (66%)	21 (34%)	0

the lamina propria ($\rho = -0.309$, $P = .023$, $\rho = 0.268$, $P = .050$, and $\rho = 0.354$, $P = .009$, respectively) (Figure 6).

3.6. Assessing the risk of disease recurrence: the predictive capability of ECM markers

Within the first year of follow-up, a total of 54 patients (86%) in the stenotic group underwent a colonoscopy, of these, 20 patients (32%) had confirmed Rutgeerts endoscopic disease recurrence (i2b) within the first year of follow-up. A total of 4 patients (6%) had a score of i3, and 2 patients (3%) had a score of i4. None of the patients had recurrent fibrostenotic disease during the 1-year follow-up. When evaluating the ability of marker values to predict recurrence based on measurements taken at inclusion, POD, and at 1, 3, and 6 months, none of the markers demonstrated predictive capacity for disease recurrence, nor did the routine markers (Table S5). Logistic regression analysis showed that smoking (OR = 1.06, 95% CI, 0.33–3.28), gender (OR = 1.86, 95% CI, 0.62–5.85), and age at resection (OR = 1.00, 95% CI, 0.97–1.04) were not significantly associated with recurrence.

4. Discussion

In this study, we described the progression of collagen ECM markers in fibrostenosing CD. We compared the data with a luminal cohort, as well as patients who served as their own controls before and after bowel resection for stenosing disease. Several known and novel markers associated with fibrosis in CD were identified.

Extracellular matrix remodeling plays a fundamental role in tissue damage and repair across various tissues and organs. This process involves alterations in the structural components of the ECM, leading to the release of fragments that reflect both degradation and synthesis. These fragments act as biomarkers of transmural disease activity, encompassing inflammation and fibrosis, which can be detected through blood tests.[7,45] To date, no biomarkers have been identified that can be utilized in clinical practice to detect fibrotic stages in CD.[21] Many of our biomarkers assessed in this study are novel and have not been previously characterized in relation to IBD phenotypes.[13]

We identified several markers that could distinguish our stenosing cohort from the luminal cohort at baseline, including PRO-C6, PRO-C3, PRO-C5, C4M, PRO-C4, PRO-C16, and PRO-C17. Among these, PRO-C5 (AUC 0.83) and PRO-C6 (AUC 0.81) emerged as the strongest individual markers. When combined, PRO-C3, PRO-C5, and PRO-C6 achieved an AUC of 0.91. These results are in line with our expectations, as PRO-C3, PRO-C5, and PRO-C6 are all associated with collagen formation and fibroblast activation, processes known to be upregulated in fibrostenotic disease.[13] Although the literature is still limited, this trend has been observed in other studies. In particular, PRO-C6 has consistently been identified as a biomarker associated with organ fibrogenesis. One study showed that C3M and C4M could differentiate between the 2 groups.[46] C3M was further confirmed in another study, which showed that C3M and PRO-C3 could distinguish the cohorts, with an even stronger signal observed when expressed as the C3M/PRO-C3 ratio; a finding we also observed in our cohort.[47] A common feature of the 2 cross-sectional studies is that the phenotype is based on historical classification rather than stenosis confirmed at the time of sampling and did not include histopathologic assessment, which may make them less comparable to our data. Our study extends previous work by combining biomarker analysis with histopathological validation of stenosis and using prospective phenotype classification based on CONSTRICT criteria.[23] A study in tissue samples showed that increased collagen VI alpha-3 chain gene and protein expression is associated with the development of complicated disease with strictures.[48] In our data, we found a difference between stenosing and luminal cases in serum for the formation marker PRO-C6, which quantifies the pro-fibrotic hormone Endotrophin on the alpha-3 chain of type VI collagen. Additionally, PRO-C6 was significantly elevated in stenotic patients with grade 3 histopathological intestinal fibrosis, correlated with the degree of submucosal fibrosis within the stenotic region. In contrast, the marker C6M, which reflects mucosal damage, was elevated compared to the luminal group. In combination with the other biomarkers, these data suggest that the stenotic group have overall an increased fibroblast activity, which is reflected by the elevated levels of PRO-C3 and PRO-C6, and less mucosal damage. This is in line with the overall notion of the disease behavior where increased ECM remodeling and collagen deposition are hallmarks of stenotic CD patients.[7] In addition, as type VI collagen is highly expressed in adipose tissue, we speculate whether the PRO-C6 biomarker, in the

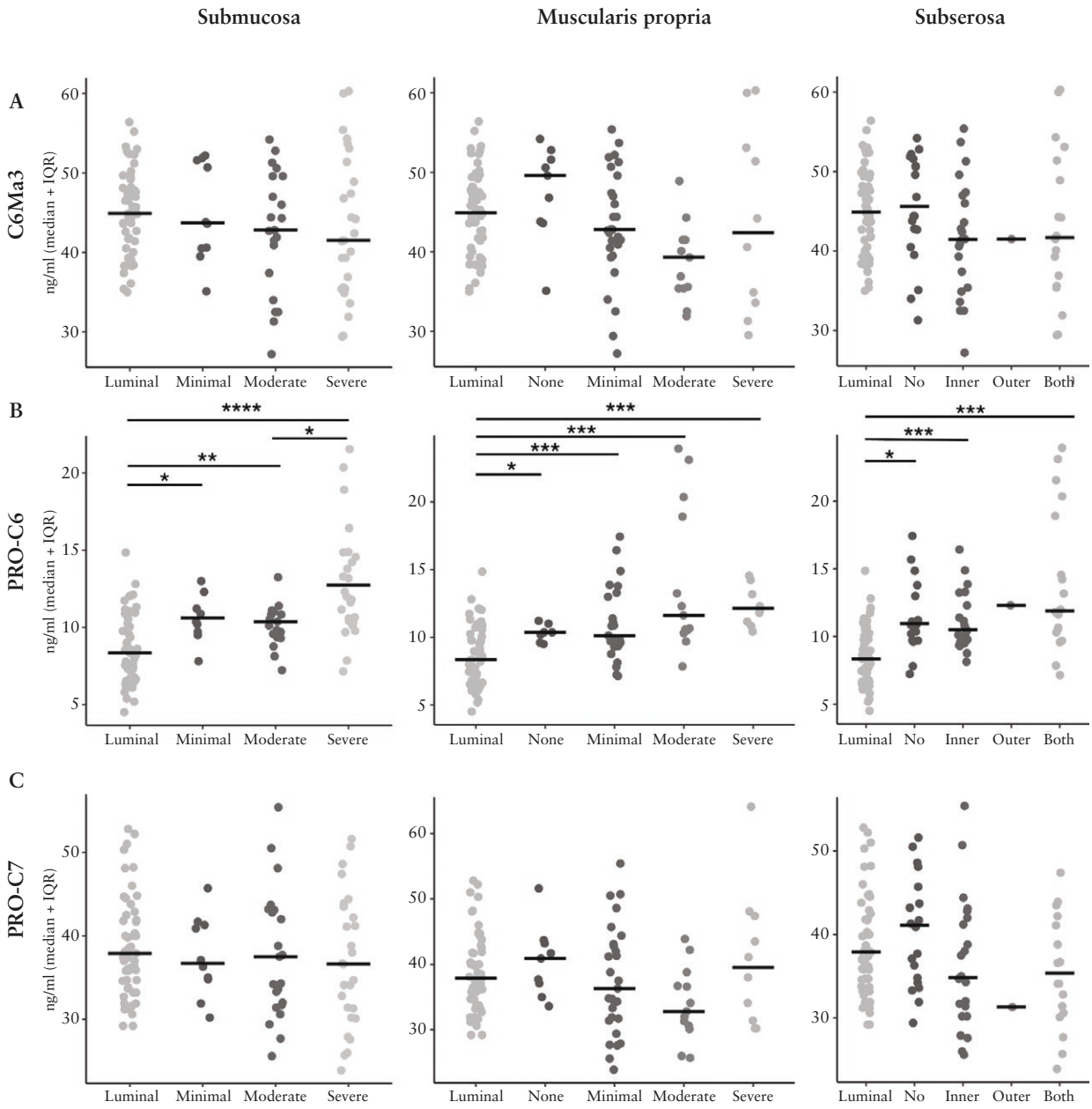


Figure 5. Scatterplots illustrating the distribution of (A) C6Ma3, (B) PRO-C6, and (C) PRO-C7 across fibrosis grades for submucosa, muscularis propria, and subserosa. Baseline serum levels for patients with luminal CD are included as comparison. The x-axis represents fibrosis severity, while the y-axis shows the corresponding biomarker values in ng/mL. Median levels are indicated with a black crossbar for each group. *$P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$. CD, Crohn's disease.

context of stenotic CD patients, could be originating from creeping fat which is also highly associated with stenosis in CD patients. Future studies should aim at proving this link between PRO-C6 and creeping fat, and further confirm PRO-C6 as a relevant biomarker for stenotic CD patients.

The post-resection process can generally be divided into 2 distinct phases. The first phase involves initial wound healing, including the physiological trauma response and the effects of surgical removal of fibrotic tissue. The second phase reflects the longer-term disease response, which may include both the consequences of fibrosis removal and the development of disease recurrence. To our knowledge, no literature exists that investigates this in IBD patients post-resection, making this study the first of its kind to adopt a systematic approach, allowing discrimination between the mentioned phases. A study has shown that mRNA expression levels of the most abundant ECM collagens revealed no difference in baseline expression between quiescent UC and controls; however, during wound healing, expression increased more in UC than in healthy controls, potentially contributing to the disease response and healing after resection.[49] One might argue that the same applies to CD. It is important to consider that research has shown ECM dysregulation in IBD differs between CD and UC, with CD exhibiting lower MMP expression than UC, making it uncertain to draw assumptions.[11]

All measured markers exhibited significant changes in serum levels on the POD compared to pre-resection, with some markers increasing and others decreasing. This may be due to the removal of the diseased intestinal segment and active fibroblast residing in the stenotic tissue, where e.g. PRO-C3 and PRO-C6 were decreased. Alternatively, this could

Table 5. Spearman's correlation was used to analyze the histological grading of fibrosis and inflammation (D'Haens score) in the stenotic areas of resected specimens, in conjunction with routine markers and our new ECM markers.

	C3M		PRO-C3		C4G		C4M	
	Roh	*P*-value	Roh	*P*-value	Roh	*P*-value	Roh	*P*-value
Transmural fibrosis burden	0.169	.188	0.061	.637	−0.034	.792	0.109	.401
Fibrosis in submucosa	0.187	.148	0.083	.527	0.045	.729	0.133	.306
Fibrosis in subserosa	0.051	.695	−0.053	.685	−0.109	.405	0.108	.405
Fibrosis in muscularis propria	0.096	.464	0.169	.191	−0.049	.707	0.029	.825
Muscularization of submucosa	0.127	.329	0.093	.474	−0.062	.637	−0.011	.932
D'Haens	0.112	.431	−0.157	.266	−0.048	.736	0.134	.343
Epithelial damage	0.049	.714	−0.159	.227	−0.137	.300	0.083	.533
Architectural damage	0.117	.399	−0.065	.643	−0.103	.459	0.117	.398
Mononuclear cells lamina propria	0.161	.244	***−0.309***	***.023***	−0.219	.111	***0.268***	***.050***
Neutrophils lamina propria	0.165	.239	−0.177	.206	0.074	.599	0.254	.663
Neutrophils in the epithelium	0.014	.921	−0.172	.224	−0.075	.597	0.080	.571
Ulcer/erosion	0.054	.677	−0.102	.429	−0.064	.623	0.081	.532
	PRO-C4		**PRO-C5**		**C6M**		**PRO-C6**	
Transmural fibrosis burden	0.099	.443	−0.178	.165	0.092	.477	0.212	.098
Fibrosis in submucosa	0.126	.333	−0.134	.303	0.171	.188	**0.304**	**.017**
Fibrosis in subserosa	0.062	.637	−0.204	.114	0.106	.415	0.248	.054
Fibrosis in muscularis propria	0.089	.497	−0.169	.192	−0.019	.885	0.085	.517
Muscularization of submucosa	0.045	.732	−0.114	.382	−0.088	.501	0.097	.456
D'Haens	0.172	.222	−0.128	.365	0.068	.634	0.122	.389
Epithelial damage	0.174	.188	0.021	.877	0.091	.491	0.068	.609
Architectural damage	0.224	.103	−0.135	.329	0.090	.517	−0.022	.873
Mononuclear cells lamina propria	***0.354***	***.009***	0.073	.600	0.132	.339	−0.060	.664
Neutrophils lamina propria	***0.304***	***.027***	−0.034	.809	0.087	.538	0.069	.619
Neutrophils in the epithelium	0.043	.762	−0.159	.259	0.013	.926	0.224	.110
Ulcer/erosion	0.175	.173	−0.088	.498	0.094	.465	0.181	.159
	C6MA3		**PRO-C7**		**PRO-C11**		**PRO-C16**	
Transmural fibrosis burden	−0.182	.156	−0.139	.281	0.004	.977	−0.099	.445
Fibrosis in submucosa	−0.082	.529	−0.041	.756	0.073	.582	−0.063	.627
Fibrosis in subserosa	−0.239	.064	−0.139	.286	−0.126	.337	−0.128	.327
Fibrosis in muscularis propria	−0.164	.206	−0.249	.053	−0.074	.574	−0.069	.599
Muscularization of submucosa	−0.071	.587	−0.118	.366	0.008	.953	−0.054	.678
D'Haens	−0.046	.748	−0.006	.964	0.003	.982	−0.087	.539
Epithelial damage	0.033	.805	−0.085	.520	−0.033	.804	0.052	.688
Architectural damage	−0.018	.896	−0.192	.164	0.012	.929	−0.053	.703
Mononuclear cells lamina propria	−0.007	.961	−0.123	.374	−0.159	.256	0.139	.314
Neutrophils lamina propria	0.054	.702	0.008	.956	0.121	.395	0.055	.695
Neutrophils in the epithelium	−0.055	.698	−0.023	.871	−0.003	.983	−0.141	.320
Ulcer/erosion	−0.005	.970	−0.021	.870	−0.046	.726	0.050	.699
	PRO-C17		**PRO-C22**		**CTX-III**			
Transmural fibrosis burden	−0.163	.207	0.133	.304	−0.067	.608		
Fibrosis in submucosa	−0.024	.853	0.176	.176	−0.086	.512		
Fibrosis in subserosa	−0.173	.183	0.102	.435	−0.152	.244		
Fibrosis in muscularis propria	−0.142	.274	0.092	.481	−0.057	.665		
Muscularization of submucosa	−0.241	.061	−0.036	.786	0.061	.639		
D'Haens	−0.073	.605	0.189	.179	***−0.276***	***.048***		
Epithelial damage	−0.121	.361	0.254	.052	***−0.285***	***.029***		
Architectural damage	−0.054	.696	0.241	.079	−0.252	.066		
Mononuclear cells lamina propria	−0.195	.158	0.212	.124	***−0.358***	***.008***		

Table 5. Continued

	C3M		PRO-C3		C4G		C4M	
	Roh	*P*-value	Roh	*P*-value	Roh	*P*-value	Roh	*P*-value
Neutrophils lamina propria	−0.165	.238	0.259	.061	−0.198	.156		
Neutrophils in the epithelium	−0.144	.310	0.169	.231	***−0.335***	***.015***		
Ulcer/erosion	−0.109	.395	0.177	.168	***−0.345***	***.006***		

Significant findings ($P < .05$) are highlighted in bold.

be attributed to the role of collagens in the wound healing response and the induced local tissue trauma, where the markers would be expected to increase postoperatively as an indicator of ECM remodeling, as seen with, for example, C4G, PRO-C11, and PRO-C22. Interestingly, the C4G is reflective of T-cell activity, which suggests increased T-cell influx/recruitment to the site of injury.[31] Several markers only showed differences from baseline to POD but not at later time points. This may indicate that they are not directly related to fibrosis levels or that their presence is more indicative of a tendency to develop a specific phenotype. It should be noted that fibrosis was identified at the oral resection margin in 32% and at the anal margin in 21% of resected specimens with macroscopically normal margins, reported as disease-free following resection. This indicates that fibrosis is more widespread than the macroscopically visible fibrosis within the stenosis itself, which could affect biomarkers reflecting fibrosis burden. For example, PRO-C3 is a well-documented fibrosis marker in liver diseases,[14] but here it does not differentiate between pre- and post-resection samples. This could suggest that PRO-C3 plays a role in initiating fibrogenesis rather than serving as a measure for the amount of established fibrosis burden. In the intestine, we can remove the stenosis via resection to some extent, which is not feasible in the same way for the liver, and thus this hypothesis cannot be tested in the liver as we do here. Fibrosis markers do not necessarily originate from the main stenotic fibrotic tissue itself; they may derive from other intestinal segments where their presence facilitates fibrosis and, consequently, stenosis formation. Further, ECM-based markers might reflect the overall remodeling of extra-intestinal tissues as well. Many aspects of this process remain poorly understood. The regulatory pattern of ECM markers observed in this study underscores the need for prospective longitudinal studies spanning a fibrosis-reducing event—such as resection—to evaluate the effectiveness of potential fibrosis markers in IBD. Without this design, several markers could have been misinterpreted as indicators of established fibrosis.

We have employed routine markers for numerous years, and they have been characterized as indicators of disease severity rather than specific markers of fibrosis.[50,51] Our findings showed that routine markers correlated with both tissue fibrosis and the level of inflammation. Nevertheless, we maintain that these routine markers cannot be classified as fibrosis markers. Given the association between fibrosis and severe disease, this correlation likely reflects the markers' ability to track disease severity, as they lack a direct relationship with fibrosis itself.[21] These findings highlight the distinctions in disease activity between luminal and stenosing disease groups. Research has shown that elevated CRP is associated with severe outcomes, while fecal calprotectin demonstrates greater sensitivity than CRP to endoscopic inflammatory burden.[52] In other studies, CRP has been demonstrated to predict disease progression in newly diagnosed patients, with elevated CRP levels linked to a more complicated disease trajectory.[53,54] Fecal calprotectin has been found to reflect, but not forecast, disease recurrence following resection.[55]

Neither the ECM markers nor the routine markers were able to predict disease recurrence within the first year post-resection. No patients developed stenosis during the first year, and most recurrences were of mild severity. In addition to the generally mild disease course, it is also possible that heterogeneity in baseline inflammatory activity or subtle differences in imaging findings not captured in this study may have influenced the lack of predictive value observed. These factors should be explored in future studies to better understand the conditions under which ECM biomarkers can predict recurrence. Consequently, a longer follow-up period would likely have led to an increase in stenosis and more severe disease, potentially enhancing the ability of biomarkers to detect these conditions. Future studies should consider extended follow-up and investigate integrative approaches combining ECM biomarkers with imaging and endoscopic findings. Moreover, using panels of biomarkers that reflect different phases of the underlying molecular processes or target distinct anatomical layers of the bowel wall, in particular ECM-related markers reflecting deeper tissue activity such as collagen remodeling in the submucosa, may improve the sensitivity and specificity of biomarker-based prediction. PRO-C3 has been examined in 4 other studies regarding treatment response[15,56–58] involving patients with a confirmed diagnosis of CD.[22] Three of these studies indicated that PRO-C3 was capable of predicting treatment response as well as long-term effects.[56–58] The difference between detecting treatment response and disease relapse lies in the definition used for endoscopic disease recurrence (Rutgeerts score ≥i2b), which includes minor superficial lesions often found during postoperative scheduled endoscopies in asymptomatic patients. In contrast, studies on disease activity primarily focus on more severe cases that lead to symptoms and are thus easier for biomarkers to detect.

Our study offers valuable insights into histology and ECM markers in CD, but some limitations should be acknowledged. First, the use of serum markers to assess ECM formation and degradation may not fully represent the intricate processes of tissue remodeling in CD, as these markers may not capture localized changes within the intestinal wall. Second, ECM markers are relatively novel in the IBD field, and their clinical relevance remains to be fully clarified. In addition, the consensus on assessing and grading intestinal fibrosis is also evolving. A precise measurement of a disease process, such as intestinal fibrosis, is therefore essential for evaluating a biomarker's true potential as a surrogate marker for this process. However, it is not feasible to perform transmural histopathological assessment of every intestinal segment as

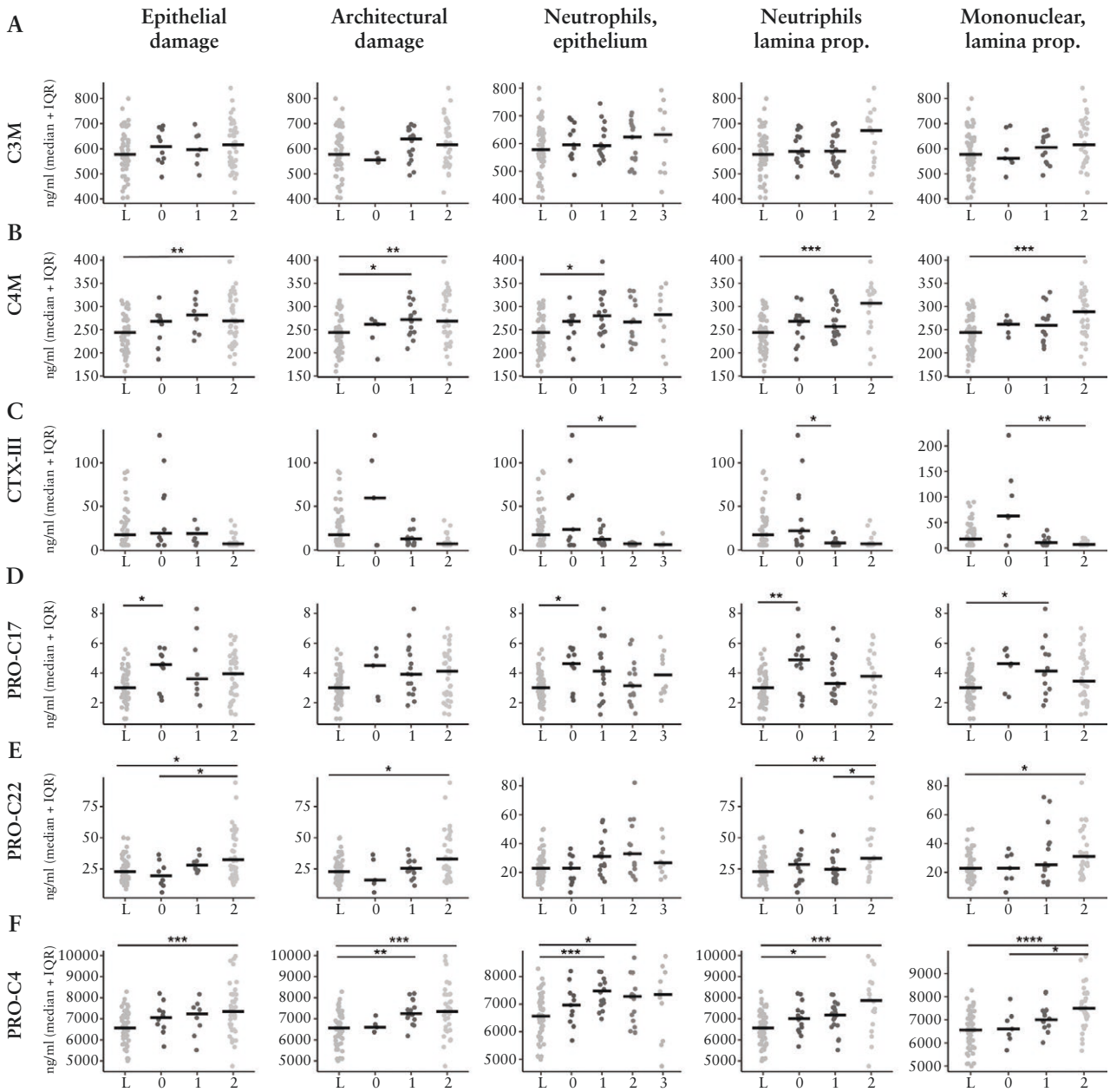


Figure 6. Scatterplots illustrating the distribution of (A) C3M, (B) C4M, (C) CTX-III, (D) PRO-C17, (E) PRO-C22, and (F) PRO-C4 across subscores of the D'Haens score. The x-axis represents grades, while the y-axis shows the corresponding biomarker values in ng/mL. Baseline serum levels for patients with luminal CD are included as comparison and annotated as "L." Median levels are indicated with a black crossbar for each group. *$P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$. CD, Crohn's disease.

a measure of total fibrosis burden, increasing the likelihood of underestimating the severity of mucosal damage or intestinal fibrosis in areas without detected disease activity at the time of inclusion in the study. Subclinical changes in intestinal tissue may be more readily detected using biomarkers, such as serum biomarkers, which reflect transmural disease stages during ECM remodeling. Although the sample size of 62 patients is noteworthy, it may still be insufficient to generalize findings across the broader CD population, highlighting the need for larger studies to validate these results. Given the number of comparisons performed, the risk of false-positive findings due to multiple testing cannot be excluded. However, this study was exploratory in nature, and the analyses were hypothesis-generating rather than confirmatory. Future validation studies in independent cohorts are warranted to confirm the observed associations. Lastly, as a prospective longitudinal cohort study, our design emphasizes the importance of extended follow-up periods. Longer follow-up would likely reveal disease recurrence more clearly and enable better differentiation with individual ECM markers. Such studies could further assess the ability of these biomarkers to predict long-term clinical outcomes and track their behavior over time in relation to disease progression or remission.

In conclusion, this study provided important insights into the role of ECM markers in fibrostenosing CD, distinguishing these markers between stenosing and luminal cohorts, where PRO-C6, PRO-C3, and PRO-C5 emerged as the best biomarkers for discriminating between stenotic and luminal CD patients. The findings suggest that ECM remodeling significantly influences tissue damage and repair processes in CD.

Despite the significant post-resection changes in marker levels, ECM markers and routine markers could not predict disease recurrence within the first year post-resection, likely due to the mild nature of most recurrences and absence of stenosis in that timeframe. While the study demonstrated clear differences in ECM marker profiles between stenotic and luminal phenotypes, consistent biomarker patterns following resection were less evident. This may reflect the complexity of postoperative tissue remodeling processes and highlights the need for longer follow-up and more refined tools to evaluate fibrosis-related disease activity in this context.

Acknowledgments

Poster presentation, ECCO, Berlin, 2025.

Author contributions

Anja Poulsen: study concept and design; acquisition of data; analysis and interpretation of data; drafting and approval of the final manuscript. Marta Sorokina Alexdóttir, Joachim Høg Mortensen, Peter-Martin Krarup, Thomai Tsapanou-Katranara: laboratory analyses; analysis and interpretation of data, critical revision, and approval of the final manuscript. Lene Buhl Riis: study concept and design; tissue handling and histological descriptions; approval of the final manuscript. Pernille Dige Ovesen, Julie Rasmussen, Mads Damsgaard Wewer, Viviane Lin, Ronja M.B. Lagström, Emilie Dahl, Marta Sorokina Alexdóttir, Annedorte Ries: study concept and design; patient inclusion and follow-up; critical revision, and approval of the final manuscript. Peter-Martin Krarup, Ismail Gögenur, Florian Rieder, Johan Burisch: study concept and design; critical revision, and approval of the final manuscript. Jakob Benedict Seidelin: study concept and design; analysis and interpretation of data; main study supervision; critical revision; approval of the final manuscript.

Funding

This work was supported by Overlæge Poul Martin Christiansen og Hustrus Foundation, Grosserer L. F. Foghts Foundation, Aase og Ejnar Danielsens Foundation (20-10-0344), and an Investigator Initiated Study grant from Janssen-Cilag A/S (CNTO1275CRD4044).

Conflicts of interest

A.P.: reports research grants and personal fees from Janssen, Takeda, and MSD, grant from Novo Nordisk, personal fees from Bristol Myers Squibb. National coordinator of studies from AbbVie, Boehringer Ingelheim, and Bristol Myers Squibb. None of these pertain to the research submitted here. M.S.A.: None. L.B.R.: Consultant fees for advisory board membership Sakura Finetek Europe. P.D.O.: None. J.R.: None. M.D.W.: reports grants from Bristol Myers Squibb, Novo Nordisk Foundation, Aage og Johanne Louis-Hansens Fond, Boehringer Ingelheim. V.L.: None. R.M.B.L.: None. M.A.-S.: None. E.D.: Speaker fee from MSD, research grants from Takeda. A.R.: None. M.P.: None. T.T.-K.: None. P.M.K.: None. I.G.: reports research grants from Pharmacosmos, Intuitive Surgical, and personal fees for congress attendance and presentations from Pharmacosmos, Intuitive Surgical, Johnson and Johnson. None of these pertain to the research submitted here. F.R.: reports consulting and advisory boards for Adnovate, Agomab, Allergan, AbbVie, Boehringer Ingelheim, Celgene/BMS, CDISC, Cowen, Galmed, Genentech, Gilead, Gossamer, Guidepoint, Helmsley, Index Pharma, Jannsen, Koutif, Mestag, Metacrine, Morphic, Organovo, Origo, Pfizer, Pliant, Prometheus Biosciences, Receptos, RedX, Roche, Samsung, Surmodics, Surrozen, Takeda, Techlab, Theravance, Thetis, UCB, Ysios, 89Bio and research funding from the NIH, Helmsley Charitable Trust, Crohn's and Colitis Foundation, UCB, Pliant, BMS, AbbVie, Pfizer, Boehringer Ingelheim, Morphic, Kenneth Rainin Foundation. J.B.: reports grants and personal fees from AbbVie, grants and personal fees from Janssen-Cilag, personal fees from Celgene, grants and personal fees from MSD, personal fees from Pfizer, grants and personal fees from Takeda, grants and personal fees from Tillotts Pharma, personal fees from Samsung Bioepis, grants, and personal fees from Bristol Myers Squibb, grants from Novo Nordisk, personal fees from Pharmacosmos, personal fees from Ferring, personal fees from Galapagos, outside the submitted work. J.H.M.: None. J.B.S.: has received research grants from Takeda, Janssen, the Danish Research Council, and the Capital Region Denmark, and is national coordinator of studies from AbbVie, Arena Pharmaceuticals, Ely Lilly, and Boehringer Ingelheim. None of these pertain to the research submitted here.

Data availability

The data underlying this article are available in the article and its online supplementary material. Additional data can be obtained from the corresponding author upon request.

Supplementary material

Supplementary material is available at *ECCO-JCC* online.

References

1. Rieder F, Mukherjee PK, Massey WJ, Wang Y, Fiocchi C. Fibrosis in IBD: from pathogenesis to therapeutic targets. *Gut*. 2024;**73**:854–866.
2. Rieder F, Fiocchi C, Rogler G. Mechanisms, management, and treatment of fibrosis in patients with inflammatory bowel diseases. *Gastroenterology*. 2017;**152**:340–350.e6.
3. Rieder F, Zimmermann EM, Remzi FH, Sandborn WJ. Crohn's disease complicated by strictures: a systematic review. *Gut*. 2013;**62**:1072–1084.
4. Sleiman J, El Ouali S, Qazi T, et al. Prevention and treatment of stricturing Crohn's disease - perspectives and challenges. *Expert Rev Gastroenterol Hepatol*. 2021;**15**:401–411.
5. Wu TH, Brandt CF, Scheike T, Burisch J, Jeppesen PB. The Natural History of Crohn's disease leading to intestinal failure: a longitudinal cohort study from 1973 to 2018. *J Crohns Colitis*. 2024;**19**. doi: 10.1093/ecco-jcc/jjae114
6. Khan S, Sebastian SA, Parmar MP, et al. Factors influencing the quality of life in inflammatory bowel disease: a comprehensive review. *Dis Mon*. 2024;**70**:101672.
7. Mortensen JH, Lindholm M, Langholm LL, et al. The intestinal tissue homeostasis–the role of extracellular matrix remodeling in inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol*. 2019;**13**:977–993.
8. Petrey AC, de la Motte CA. The extracellular matrix in IBD: a dynamic mediator of inflammation. *Curr Opin Gastroenterol*. 2017;**33**:234–238.
9. Theocharis AD, Manou D, Karamanos NK. The extracellular matrix as a multitasking player in disease. *FEBS J*. 2019;**286**:2830–2869.

10. Hu WT, Fang TY, Chen XQ. Identification of differentially expressed genes and miRNAs for ulcerative colitis using bioinformatics analysis. *Front Genet*. 2022;**13**:914384.
11. Biel C, Faber KN, Bank RA, Olinga P. Matrix metalloproteinases in intestinal fibrosis. *J Crohns Colitis*. 2024;**18**:462–478.
12. Pehrsson M, Mortensen JH, Manon-Jensen T, Bay-Jensen AC, Karsdal MA, Davies MJ. Enzymatic cross-linking of collagens in organ fibrosis - resolution and assessment. *Expert Rev Mol Diagn*. 2021;**21**:1049–1064.
13. Poulsen A, Ovesen PD, Lu C, et al. Serum extracellular matrix molecules and their fragments as biomarkers of inflammation and fibrosis in inflammatory bowel diseases: a systematic review. *J Crohns Colitis*. 2024;**18**:1894–1934.
14. Mak AL, Lee J, van Dijk AM, et al. Systematic review with meta-analysis: diagnostic accuracy of Pro-C3 for hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Biomedicines*. 2021;**9**:1920.
15. van Haaften WT, Mortensen JH, Dige AK, et al. Serological biomarkers of tissue turnover identify responders to anti-TNF therapy in Crohn's disease: a pilot study. *Clin Transl Gastroenterol*. 2020;**11**:e00217.
16. Bourgonje AR, Sorokina Alexdottir M, Otten AT, et al. Serological biomarkers of Type I, III, and IV collagen turnover are associated with the presence and future progression of stricturing and penetrating Crohn's disease. *United Eur Gastroenterol J*. 2022;**10**:285.
17. Bettenworth D, Bokemeyer A, Baker M, et al.; Stenosis Therapy and Anti-Fibrotic Research (STAR) Consortium. Assessment of Crohn's disease-associated small bowel strictures and fibrosis on cross-sectional imaging: a systematic review. *Gut*. 2019;**68**:1115–1126.
18. Lu C, Rosentreter R, Delisle M, et al.; Stenosis Therapy and Anti-Fibrotic Research (STAR) consortium. Systematic review: defining, diagnosing and monitoring small bowel strictures in Crohn's disease on intestinal ultrasound. *Aliment Pharmacol Ther*. 2024;**59**:928–940.
19. Gordon IO, Bettenworth D, Bokemeyer A, et al.; Stenosis Therapy and Anti-Fibrotic Research (STAR) Consortium. International consensus to standardise histopathological scoring for small bowel strictures in Crohn's disease. *Gut*. 2021;**71**:479–486.
20. Gordon IO, Bettenworth D, Bokemeyer A, et al. Histopathology scoring systems of stenosis associated with small bowel Crohn's disease: a systematic review. *Gastroenterology*. 2019;**158**:137.
21. Steiner CA, Berinstein JA, Louissaint J, et al.; Stenosis Therapy and Anti-Fibrotic Research (STAR) Consortium. Biomarkers for the prediction and diagnosis of fibrostenosing Crohn's disease: a systematic review. *Clin Gastroenterol Hepatol*. 2022;**20**:817–846.e10.
22. Maaser C, Sturm A, Vavricka SR, et al.; European Crohn's and Colitis Organisation [ECCO] and the European Society of Gastrointestinal and Abdominal Radiology [ESGAR]. ECCO-ESGAR Guideline for diagnostic assessment in IBD Part 1: initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis*. 2019;**13**:144–164.
23. Rieder F, Bettenworth D, Ma C, et al. An expert consensus to standardise definitions, diagnosis and treatment targets for anti-fibrotic stricture therapies in Crohn's disease. *Aliment Pharmacol Ther*. 2018;**48**:347–357.
24. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol*. 2005;**19**:5A–36A.
25. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc*. 2004;**60**:505–512.
26. Rutgeerts P, Hiele M, Geboes K, et al. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. *Gastroenterology*. 1995;**108**:1617–1621.
27. Leeming D, He Y, Veidal S, et al. A novel marker for assessment of liver matrix remodeling: an enzyme-linked immunosorbent assay (ELISA) detecting a MMP generated type I collagen neo-epitope (C1M). *Biomarkers*. 2011;**16**:616–628.
28. Barascuk N, Veidal SS, Larsen L, et al. A novel assay for extracellular matrix remodeling associated with liver fibrosis: an enzyme-linked immunosorbent assay (ELISA) for a MMP-9 proteolytically revealed neo-epitope of type III collagen. *Clin Biochem*. 2010;**43**:899–904.
29. Nielsen MJ, Nedergaard AF, Sun S, et al. The neo-epitope specific PRO-C3 ELISA measures true formation of type III collagen associated with liver and muscle parameters. *Am J Transl Res*. 2013;**5**:303–315.
30. Sand JM, Larsen L, Hogaboam C, et al. MMP mediated degradation of type IV collagen alpha 1 and alpha 3 chains reflects basement membrane remodeling in experimental and clinical fibrosis--validation of two novel biomarker assays. *PLoS One*. 2013;**8**:e84934.
31. Jensen C, Sinkeviciute D, Madsen DH, et al. Granzyme B degraded Type IV collagen products in serum identify melanoma patients responding to immune checkpoint blockade. *Cancers (Basel)*. 2020;**12**:2786.
32. Leeming DJ, Nielsen MJ, Dai Y, et al. Enzyme-linked immunosorbent serum assay specific for the 7S domain of Collagen Type IV (P4NP 7S): A marker related to the extracellular matrix remodeling during liver fibrogenesis. *Hepatol Res*. 2012;**42**:482–493.
33. Vassiliadis E, Veidal SS, Simonsen H, et al. Immunological detection of the type V collagen propeptide fragment, PVCP-1230, in connective tissue remodeling associated with liver fibrosis. *Biomarkers*. 2011;**16**:426–433.
34. Nedergaard A, Sun S, Karsdal MA, et al. Type VI collagen turnover-related peptides-novel serological biomarkers of muscle mass and anabolic response to loading in young men. *J Cachexia Sarcopenia Muscle*. 2013;**4**:267–275.
35. Sun S, Henriksen K, Karsdal MA, et al. Collagen Type III and VI turnover in response to long-term immobilization. *PLoS One*. 2015;**10**:e0144525.
36. Veidal SS, Karsdal MA, Vassiliadis E, et al. MMP mediated degradation of type VI collagen is highly associated with liver fibrosis—identification and validation of a novel biochemical marker assay. *PLoS One*. 2011;**6**:e24753.
37. Nissen NI, Kehlet S, Johansen AZ, et al. Noninvasive prognostic biomarker potential of quantifying the propeptides of Type XI collagen alpha-1 chain (PRO-C11) in patients with pancreatic ductal adenocarcinoma. *Int J Cancer*. 2021;**149**:228–238.
38. Jensen C, Nielsen SH, Mortensen JH, et al. Serum type XVI collagen is associated with colorectal cancer and ulcerative colitis indicating a pathological role in gastrointestinal disorders. *Cancer Med*. 2018;**7**:4619–4626.
39. Crespo-Bravo M, Thorlacius-Ussing J, Nissen NI, et al. Levels of type XVII collagen (BP180) ectodomain are elevated in circulation from patients with multiple cancer types and is prognostic for patients with metastatic colorectal cancer. *BMC Cancer*. 2023;**23**:949.
40. Madsen EA, Thorlacius-Ussing J, Nissen NI, et al. Type XXII collagen complements fibrillar collagens in the serological assessment of tumor fibrosis and the outcome in pancreatic cancer. *Cells*. 2022;**11**:3763.
41. Pehrsson M, Manon-Jensen T, Sun S, et al. An MMP-degraded and cross-linked fragment of type III collagen as a non-invasive biomarker of hepatic fibrosis resolution. *Liver Int*. 2022;**42**:1605–1617.
42. D'Haens GR, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology*. 1998;**114**:262–267.
43. De Voogd FA, Mookhoek A, Gecse KB, et al. Systematic review: histological scoring of strictures in Crohn's disease. *J Crohns Colitis*. 2020;**14**:734–742.
44. Magro F, Sabino J, Rosini F, et al. ECCO position on harmonisation of Crohn's disease mucosal histopathology. *J Crohns Colitis*. 2022;**16**:876–883.
45. Pehrsson M, Alexdóttir MS, Karsdal MA, Thakker P, Mortensen JH. Novel fibro-inflammatory biomarkers associated with disease

activity in patients with Crohn's disease. *Expert Rev Gastroenterol Hepatol*. 2023;**17**:575–587.
46. Bourgonje AR, Alexdottir MS, Otten AT, et al. Serological biomarkers of type I, III and IV collagen turnover are associated with the presence and future progression of stricturing and penetrating Crohn's disease. *Aliment Pharmacol Ther.* 2022;**56**:675–693.
47. van Haaften WT, Mortensen JH, Karsdal MA, Bay-Jensen AC, Dijkstra G, Olinga P. Misbalance in type III collagen formation/degradation as a novel serological biomarker for penetrating (Montreal B3) Crohn's disease. *Aliment Pharmacol Ther.* 2017;**46**:26–39.
48. Lin SN, Musso A, Wang J, et al. Human intestinal myofibroblasts deposited collagen VI enhances adhesiveness for T cells—a novel mechanism for maintenance of intestinal inflammation. *Matrix Biol.* 2022;**113**:1–21.
49. Seidelin JB, Bronze M, Poulsen A, et al. Non-TGFβ profibrotic signaling in ulcerative colitis after in vivo experimental intestinal injury in humans. *Am J Physiol Gastrointest Liver Physiol.* 2024;**327**:G70–G79.
50. Li L, Xu P, Zhang Z, Zhou X, Chen C, Lu C. Platelets can reflect the severity of Crohn's disease without the effect of anemia. *Clinics (Sao Paulo)*. 2020;**75**:e1596.
51. Dranga M, Boiculese LV, Popa IV, et al. Anemia in Crohn's disease—the unseen face of inflammatory bowel disease. *Medicina (Kaunas)*. 2021;**57**:1046.
52. Mosli MH, Zou G, Garg SK, et al. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol.* 2015;**110**:802–19; quiz 820.
53. Bettenworth D, Nowacki TM, Cordes F, Buerke B, Lenze F. Assessment of stricturing Crohn's disease: current clinical practice and future avenues. *World J Gastroenterol.* 2016;**22**:1008–1016.
54. Kwon JH, Im JP, Ye BD, et al. Disease phenotype, activity and clinical course prediction based on C-reactive protein levels at diagnosis in patients with Crohn's disease: results from the CONNECT study. *Gut Liver*. 2016;**10**:595–603.
55. Tham YS, Yung DE, Fay S, et al. Fecal calprotectin for detection of postoperative endoscopic recurrence in Crohn's disease: systematic review and meta-analysis. *Therap Adv Gastroenterol.* 2018;**11**:1756284818785571.
56. Roblin X, Serone A, Yoon OK, et al. Effects of JAK1-preferential inhibitor filgotinib on circulating biomarkers and whole blood genes/pathways of patients with moderately to severely active Crohn's disease. *Inflamm Bowel Dis*. 2022;**28**:1207–1218.
57. Alexdottir MS, Bourgonje AR, Karsdal MA, et al. Serological biomarkers of extracellular matrix turnover and neutrophil activity are associated with long-term use of vedolizumab in patients with Crohn's disease. *Int J Mol Sci* . 2022;**23**:8137.
58. Alexdottir MS, Bourgonje AR, Karsdal MA, et al. Serological biomarkers of intestinal collagen turnover identify early response to infliximab therapy in patients with Crohn's disease. *Frontiers in Medicine*. 2022;**9**:933872.