Association Between Microscopic Lesions at Ileal Resection Margin and Recurrence After Surgery in Patients With Crohn's Disease

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BACKGROUND AND AIMS: **Different types of histologic lesions at the ileal margin, detected by histology, have been associated with increased rates of recurrence after ileocaecal surgery in patients with Crohn's disease (CD). We aimed to characterize histologic features of the ileal margin and to evaluate their association with disease recurrence.**

METHODS: **We collected histologic data from 211 patients with ileal or ileocolonic CD who underwent ileocolonic resections at hospitals in France from September 2010 through December 2016. Ileal margins were analyzed. Early endoscopic recurrence was defined by a Rutgeerts score of i2 or more, 6 months after surgery. We also collected data from 10 adults with healthy ileum who underwent ileocecal resection for colonic tumors (controls). Clinical relapse was defined by CD-related symptoms confirmed by imaging, endoscopy, therapy intensification, CD-related complication, or subsequent surgery.**

RESULTS: **Six months after surgery, 49% of patients had endoscopic recurrence; 5 years after surgery, 57% of patients had clinical relapse. Ileal margins were macroscopically affected in 20.9% of patients. CD transmural lesions at the margin (defined by mucosal ulceration or cryptitis, submucosal fibrosis and lymphoplasmacytic infiltrate of the subserosa) were observed in 13.6% of patients. Endoscopic recurrence was observed in 75% of patients with CD transmural lesions vs 46% of patients without (P =.005). In multivariate analysis, CD transmural lesions at the margin were independently associated with early endoscopic recurrence (OR, 3.83; 95% CI, 1.47-11.05; P =.008) and clinical recurrence (OR 2.04; 95% CI, 1.09-3.99; P =.026).**

CONCLUSION: **In patients with CD, transmural lesions at the ileal margin were associated with an increased risk of post-operative recurrence. Histologic features of the ileal margin should be included in making decisions about post-operative therapy.**

Keywords: REMIND Trial; Outcome; Prognostic Factor; IBD Surgery.

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Abbreviations used in this paper: CD, Crohn's disease; IQR, interquartile range; MP, myenteric plexitis; OR, odds ratio; TNF, tumor necrosis factor.

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Despite recent advances in the medical management of Crohn's disease (CD), more than two-thirds of patients still require intestinal resection during the course of their disease.[1–3] Furthermore, surgery is not curative and postoperative recurrence occurs in the majority of patients. Early postoperative endoscopic recurrence, which is mainly observed at the anastomosis or in the neoterminal ileum, precedes clinical recurrence and its severity predicts the long-term clinical outcome.[4] Several risk factors have been reported to increase postoperative recurrence, including active smoking, penetrating disease, history of perianal disease, previous intestinal surgery, and male sex.[5–7] There is still a need for factors that can accurately risk-stratify patients.[8]

Macroscopically involved ileal margin could increase postoperative recurrence risk but this assumption was pushed aside in 1996 by Fazio et al[9] with the results of a randomized controlled trial showing no benefit of extended resection (clearance of 12 cm vs 2 cm of normal ileal margin). Retrospective studies showed conflicting results regarding the impact of CD microscopic lesions on the ileal margin (mainly granuloma or active inflammation) on postoperative recurrence.[10–13] Several publications have described an association between plexitis and endoscopic, clinical, or surgical recurrence. Plexitis is classically defined by the presence of inflammatory cells in the myenteric or submucosal plexi.[14] There were diverse definitions of plexitis and various endpoints were used.[15–21] More recently, a cohort analysis of consecutive CD patients undergoing a first ileocaecal resection in 2 specialized centers found that microscopic involvement of the ileal margin, with the presence of granuloma, ulcers or transmural inflammation, was an independent risk factor of clinical and surgical recurrence.[22]

The impact of CD lesions at the proximal ileal margin on recurrence is, thus, still unclear. Our aims were to characterize the microscopic involvement of the proximal ileal margin and to study its predictive value on rates of endoscopic recurrence 6 months after surgery, clinical relapse, and surgery during the long-term follow-up. We performed this study on a well-characterized prospective multicenter cohort of CD patients followed from surgery.[7]

What You Need to Know

Background

We aimed to characterize histologic features of the ileal margin in patients undergoing surgery for Crohn's disease and to evaluate their association with recurrence.

Findings

In patients with Crohn's disease, transmural lesions at the ileal margin were associated with increased risk of postoperative disease recurrence.

Implications for Patient Care

Histologic features of the ileal margin should be included when physicians make decisions about postoperative therapy.

Materials and Methods

Postoperative Cohort and Patient Selection

The present work was undertaken on a prospective multicenter study performed by the REMIND group that aimed at identifying predictors of early postoperative endoscopic recurrence.[7] Patients were included between September 2010 and December 2016. Inclusion criteria were: >18 years of age, ileal or ileocolonic CD and indication of CD-related intestinal surgery (ileocolonic resection). A postoperative treatment was recommended according to a preestablished algorithm, based on the following risk factors: current smoking, previous bowel resection, penetrating phenotype, and active perianal disease (detailed in 7). All patients had, 6 months after surgery, a colonoscopy to assess the endoscopic recurrence according to the Rutgeerts score.[23] From this cohort, we selected patients who had a paraffin-embedded ileal margin sample available for histological analysis. Margins with poor quality paraffin embedding were excluded. All patients provided an informed written consent. The study was approved by AFFSAPS (IDRCB: 2009-A00205-52) and the French Ethic Committee - Hôpital Saint-Louis (CPP 2009/17), and declared to ClinicalTrials.gov (NCT03458195).

To test CD-related histological lesions' specificity, 10 control adults with a healthy ileum who underwent ileocaecal resection for colonic tumor constituted a control group for the histological analysis. All control patients provided an approval consent form.

Data Collection

Clinical parameters were collected prospectively at time of surgery and at postoperative endoscopy. They included demographical data (sex, age at diagnosis, age at surgery, disease duration at surgery, smoking status at surgery), clinical data, complication and surgical indication, anoperineal lesions, previous history of intestinal resection, extradigestive manifestations, treatment history including previous exposure to immunosuppressants (azathioprine, 6-mercaptopurine, or methotrexate) and anti-tumor necrosis factor (TNF) agents.

An endoscopy was performed 6 months after surgery. Endoscopic data were collected prospectively. All lesions were reported in all segments, including the anastomotic region and the neoterminal ileum. The physician who performed the colonoscopy evaluated the Rutgeerts score for each patient. Two physicians (M.A. and C.A.) checked the colonoscopy's report blinded to histological analysis and postoperative treatment.

Long-term data of patients with a follow-up of at least 18 months were collected retrospectively. They included clinical data (smoking behavior, type and date of clinical symptoms, type and date of CD related complications: intra-abdominal abscess, occlusive manifestation, anoperineal lesion, and death), treatment data (modification or optimization with initiation and cessation date for each drug), endoscopic data (date and results of colonoscopy performed during the follow-up), imaging data (date and results of magnetic resonance enterography or computed tomography scan performed during the follow-up), and surgical data (date and type of subsequent surgery performed).

Histopathological Analysis

Paraffin blocks of the ileal margin were cut into a full-thickness 3-μm section and stained with hematoxylin eosin saffron. Each section was jointly analyzed by 2 expert pathologists (D.C.H. and C.G.) blinded to the baseline detailed clinical data and to the postoperative outcomes of the patients. An analysis grid was built to assess CD-related lesions affecting each intestinal wall layer (mucosa, submucosa, and subserosa or muscularis).

Each of these lesions was considered as binary variable except for plexitis, for which 3 degrees were defined according to Ferrante et al.[15] Ileal margins from the control group (10 patients with healthy ileum without CD) were similarly examined for the same histologic lesions.

For macroscopic evaluation, we collected the length of ileal resection for each patient (in centimeters) and the distance from the ileal margin to the first macroscopic CD mucosal ulceration noticed by pathologist on opened specimen (in centimeters). The margin was defined as "macroscopically affected" if CD's mucosal ulcerations were noticed on the ileal margin.

Evaluated Outcomes

Postoperative endoscopic recurrence was evaluated 6 months after surgery. Endoscopic recurrence was defined as a Rutgeerts score $\geq$ i2.

Clinical recurrence was defined by (1) CD-related clinical manifestations confirmed either by endoscopy (Rutgeerts score $\geq$ i2 or severe colonic lesions in 1 of the follow-up colonoscopies), imaging (active lesions of the small bowel or colon on an examination performed by the center referring radiologist) or therapeutic intensification (treatment optimization or drug switch); (2) CD-related complications (intra-abdominal abscess or occlusive manifestation); or (3) CD-related subsequent surgery.

Surgical recurrence was defined by a further CD-related surgery at the anastomotic site (subsequent ileocolonic resection or stricturoplasty).

Table 1. Patient Characteristics at Time of Surgery (n = 211)

Men	92 (44)
Age, y	36.3 (30.7–47.5)
Age at Crohn's disease diagnosis (Montreal classification)	
- ≤16 y (A1)	19 (9)
- 17 –40 y (A2)	170 (81)
- >40 y (A3)	22 (10)
Disease duration, y	6.3 (1.8–11.9)
Time between resection and colonoscopy, mo	6.8 (6.1–8.4)
Smoking	
- Active smoker	67 (32)
- Smoking cessation at surgery	13 (6)
- Nonsmoker	131 (62)
Previous intestinal resection	38 (18)
- <10 y	14 (7)
Number of previous resection(s)	
- 0	173 (82)
- 1	27 (13)
- 2	7 (3)
- 3	4 (2)
Surgical indication	
- Stricturing complication	115 (55)
- Penetrating complication	83 (39)
- Failure of drug therapy	13 (6)
Disease location (Montreal classification)	
- Ileal (L1)	132 (63)
- Ileocolonic (L3)	79 (37)
- Anoperineal lesion	50 (24)
Extra-digestive symptoms	
- Joint manifestations	40 (19)
- Skin manifestations	12 (6)
- Eye manifestations	4 (2)
Previous exposure to anti-TNF therapy	134 (64)
Anti-TNF therapy within 3 mo before surgery	107 (51)
Previous exposure to thiopurines	145 (69)
Thiopurines within 3 mo before surgery	60 (28)

Values are n (%) or median (interquartile range).
TNF, tumor necrosis factor.

Statistical Analyses

Descriptive statistics were calculated for all quantitative variables as medians with their respective interquartile ranges (IQRs). Correlation between histology and recurrence, for each item of the analysis grid, was evaluated by both univariate and multivariate analyses. Chi-square and logistic regression were used for binary variables. For continuous data (lengths in centimeters), the Mann-Whitney test was used. Sensitivity, specificity, positive and negative predictive values were calculated using standard formulas. Spearman's rank correlation test was used when necessary to evaluate the correlation between variables.

For both clinical and surgical recurrence, Kaplan-Meier survival curves and crude log-rank tests were calculated for all variables (histological items and clinical data at baseline) before survival multivariate analysis. A multivariate Cox model combining demographical data (sex and age) and variables significantly associated with clinical or surgical recurrence in univariate analyses was built.

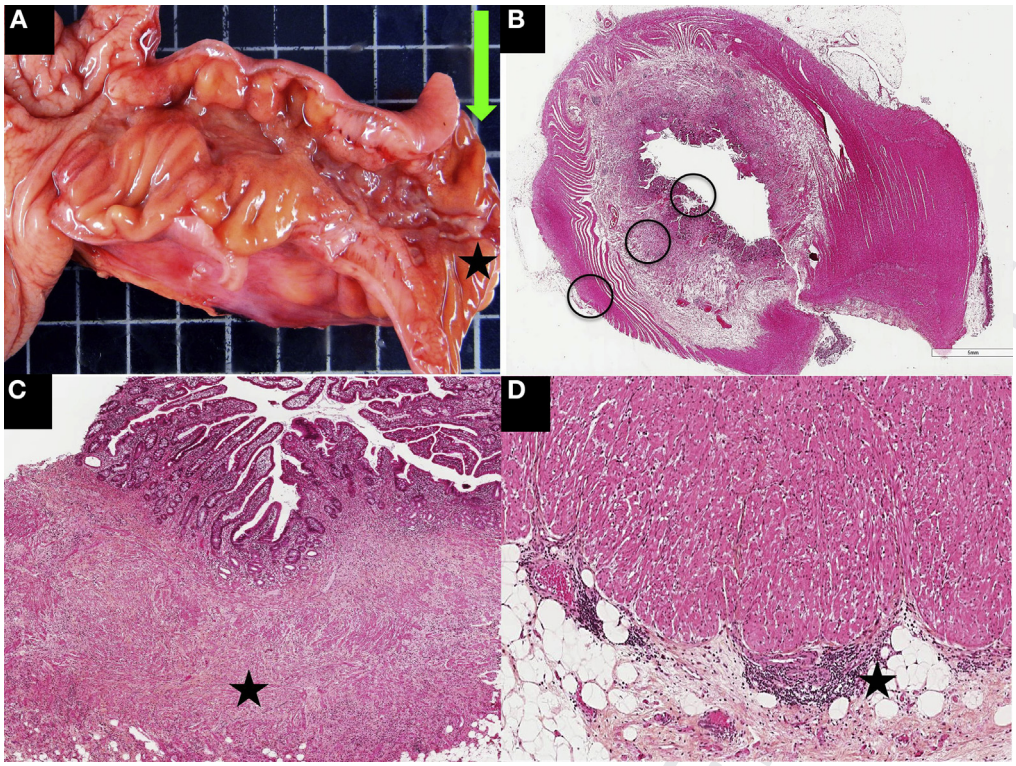

Figure 1. Pathological analysis of the whole ileal margin on surgical specimen and on section stained by hematoxylin eosin saffron. (*A*) Macroscopic view of an opened ileocecal resection specimen with mucosal ulcerations visible on ileal proximal margin (arrow). (*B*) Microscopic view of the whole ileal margin section showing a bowel wall with Crohn's disease-related lesions affecting the 3 layers (mucosal, submucosal, and subserosal layers in circles). (*C*) Fibrosis observed in the submucosa. (*D*) Lymphoplasmocytic infiltrate observed in the subserosa.

All statistical analyses were 2-tailed and a *P* value of $<.05$ was considered as statistically significant. To perform these analyses, R software version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism (GraphPad Software, San Diego, CA) were used.

Results

Patients' Characteristics at Surgery and During Follow-Up

Two hundred and twenty five patients were included.[7] Ileal margins were not technically exploitable for 14. In total, ileal margins were analyzed in 211 patients and 206 ileal margins had a complete transmural histological evaluation (Supplementary Figure 1).

Median age at surgery was 36.3 (IQR, 30.7–47.5) years and median disease duration was 6.3 (IQR, 1.8–11.9) years. Ninety-two (44%) patients were men, 67 (32%) were active smokers, and 38 (18%) had a previous ileocaecal resection in their past medical history (Table 1). Postoperative treatment was given to 102 (48%) patients, including an anti-TNF treatment in 52 (25%) patients.

Postoperative endoscopy was performed in all patients with a median time between resection and colonoscopy of 6.8 months. One hundred and four (49%) patients had an endoscopic recurrence (ie, Rutgeerts score $\geq$i2) and 43 (20%) patients had severe lesions (ie, i3 or i4). One hundred and seven (51%) patients had no endoscopic recurrence, including 68 (32%) without any lesion (i0).

Long-term follow-up (more than 18 months) was available in 168 (75%) patients. Baseline characteristics, postoperative management and endoscopic recurrence rates of these 168 patients did not differ significantly from the whole cohort.

Median follow-up was 3.8 (IQR, 2.55–5.41) years. Seventy-five (45%) patients experienced clinical relapse during the follow-up. Cumulative clinical recurrence rate was 5%, 37%, and 57% at 1, 3, and 5 years respectively. At the end of the follow-up, 4 (2%) patients underwent a CD-related subsequent surgery (3 ileocolonic resections, 1 stricturoplasty).

Histopathological Analysis of Ileal Margin

Median ileal resection length was 19 (IQR, 12–27) cm. The ileal margin was considered as macroscopically affected by the pathologist in 44 (20.9%) patients (Figure 1). Among them, 41 (93.2%) presented with at least 1 mucosal lesion in histology. The median distance between macroscopic ulceration (noticed by the pathologist in 178 patients) and the ileal margin was 4 (IQR, 1–8) cm.

Microscopically, grade 1 myenteric plexitis (MP) was found on the ileal margin of the 10 control patients revealing CD-unrelated lesion (discarded from the final grid). Among the 211 patients studied, 65 (30.8%) had a normal ileal margin and 146 (69.2%) had at least 1 CD lesion (Table 2). The most affected layer of the bowel wall was the mucosa (111 patients (52.6%)). Sixty patients (28.4%) had mucosal ulceration or cryptitis

Table 2. Pathological Data After Examination of the Ileal Margin of the 211 Patients and Correlation of Each Histological Lesion With Endoscopic Recurrence

	Prevalence (Affected/Analyzed)	Endoscopic Recurrence (%)	*P*
Microscopic data			
Normal margin	65/211 (30.8)	46.2	.54
Margin affected	146/211 (69.2)	50.7	.54
Mucosal layer	111/211 (52.6)	51.4	.58
Granuloma	18/211 (8.5)	55.6	.58
Eosinophilic Infiltrate	68/182 (37.4)	48.5	.88
Ulceration/cryptitis	60/211 (28.4)	58.3	.09[a]
Villous atrophy	20/211 (9.5)	55.0	.59
Pyloric metaplasia	43/211 (20.5)	51.2	.78
Submucosal layer	104/210 (49.3)	52.9	.30
Neutrophilic infiltrate	16/210 (7.6)	62.5	.27
Lymphoplasmocytic infiltrate	84/210 (40.0)	53.6	.31
Plexitis	81/210 (38.6)	48.1	.79
Nervous hyperplasia	17/210 (8.1)	52.9	.75
Fibrosis	71/210 (33.8)	59.1	.04[a]
Subserosal and muscularis layer	92/211 (43.6)	50.5	.78
Lymphoplasmocytic infiltrate	58/206 (28.2)	60.3	.04[a]
Fibrosis	22/206 (10.7)	59.1	.33
Grade 2 MP	74/211 (35.1)	50.0	.77
Grade 3 MP	38/211 (18.0)	50.0	.92
Macroscopic data			
Margin macroscopically affected	44/211 (20.9)	56.1	.31
	Nonrecurrent patients	Recurrent patients	
Length of ileal resection, cm	19.0	18.8	.96
Distance between first macroscopic ulceration and ileal margin, cm	4.0	4.0	.43

Values are n/n (%), unless otherwise indicated.
The margin is "affected" if at least 1 histological lesion is observed in 1 of the bowel wall layer. Margin macroscopically affected is defined as mucosal ulceration observed by the pathologist on the ileal margin when opening the surgical specimen. Granuloma is a nest of at least 5 epithelioid cells in the wall; eosinophilic infiltrate is a nest of at least 50 eosinophils in the lamina propria at 400× magnification; villous atrophy is an extensive subtotal/total villous atrophy; pyloric metaplasia involves metaplastic glands of the ileal mucosa; ulceration/cryptitis is acute inflammation (neutrophils) in epithelium or crypt; neutrophilic infiltrate is a nest of at least 50 neutrophils in the submucosae at 400× magnification; lymphoplasmocytic infiltrate is a nest of at least 50 lymphocytes or plasma cells at 400× magnification; plexitis involves inflammatory cells around or inside submucosal or myenteric plexus as defined previously.[14] Grade 1 MP is at least 1 but <4 immune cells within or contiguous to the plexus, grade 2 MP is at least 4 but <10 immune cells, and grade 3 MP is at least 10 immune cells. Nervous hyperplasia involves enlarged nervous bundles visible at 100× magnification; fibrosis involves significant collagen deposits in intestinal layer visible at 100× magnification. MP, myenteric plexitis.
[a]•••.

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indicative of CD activity. The submucosal layer was affected in 104 (49.3%) patients, 16 (7.8%) with neutrophils infiltrate (all associated with active mucosal lesion). The most frequent submucosal lesions were lymphoplasmacytic infiltrate (40%) and fibrosis (33.8%). All patients with submucosal plexitis (38.6%) had concomitant lymphoplasmacytic infiltrate. The subserosa or muscularis (plexitis included) were affected in 92 (44.7%) patients. The subserosa was affected in 64 (31.1%) patients (Supplementary Figure 2).

Patients with at least 1 lesion of each layer frequently presented grade 2 or 3 MP: 21 (55%) and 12 (33%) patients, respectively. Interestingly, plexitis (either submucosal or myenteric) was the only lesion found in 6 (3%) patients.

The presence of microscopic lesions was inversely correlated with the distance between ileal margin and macroscopic ulceration: patients with transmural lesions had a shorter disease-free margin when compared with patients with 2, 1, or 0 of the 3 histological features defining transmural impairment (Spearman's rank correlation test: r = −0.476; $P < .001$) (Supplementary Figure 3).

Correlation With Endoscopic Recurrence

The ileal resection length, the macroscopic involvement of the ileal margin and the distance between the ileal margin and the first macroscopic CD ulceration were not correlated with endoscopic recurrence ($P = .96$; $P = .31$, and $P = .43$ respectively).

In univariate analysis, two histological lesions were significantly associated with endoscopic recurrence: submucosal fibrosis (recurrence rate: 59.1% vs. 44.6%; odds ratio [OR], 1.80; 95% confidence interval [CI], 1.01–3.21; $P = .04$) and lymphoplasmacytic infiltrate of the subserosa (recurrence rate: 60.3% vs. 44.6%; OR, 1.89; 95%CI, 1.01–3.51; $P = .04$) (Table 2). A trend toward recurrence was also found in patients displaying mucosal ulceration or cryptitis indicative of active CD

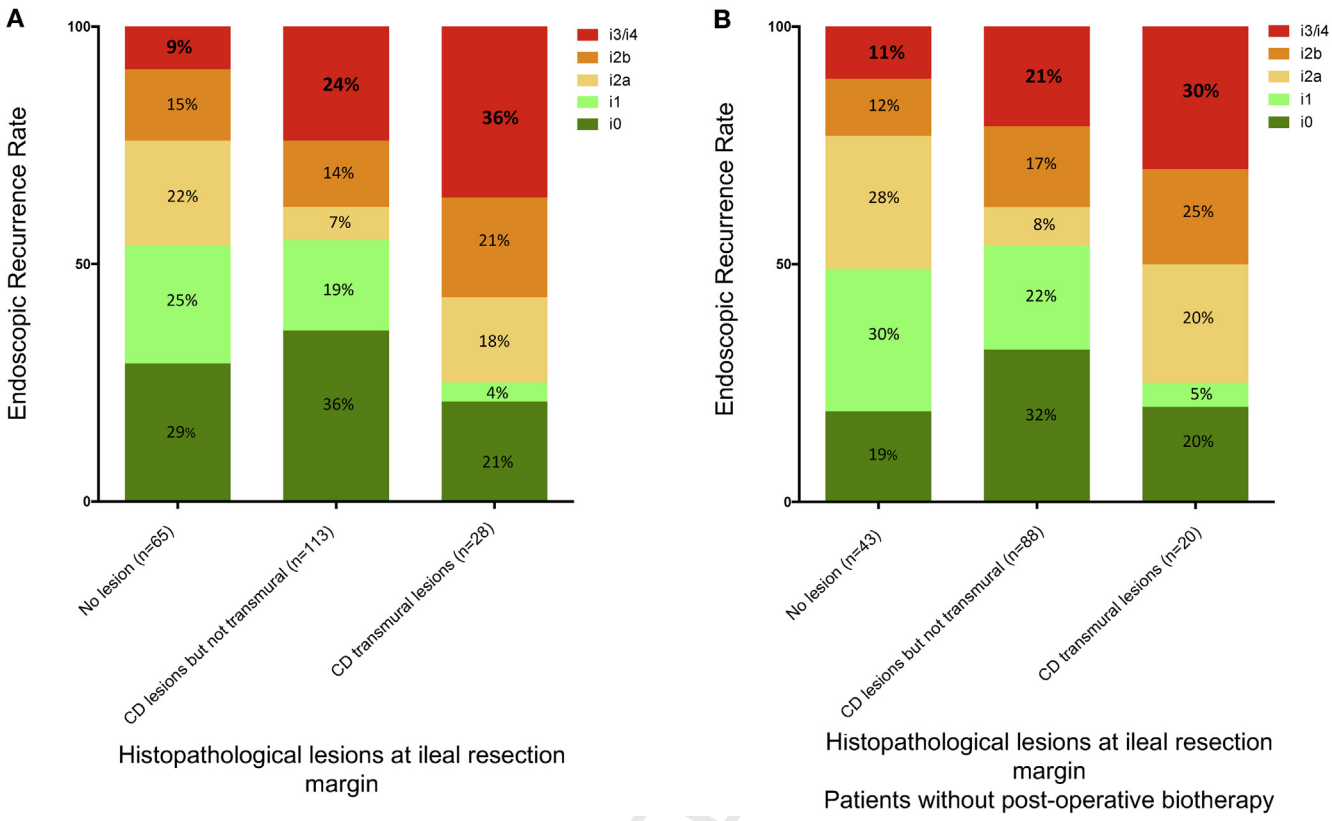

Figure 2. Endoscopic recurrence rates depending on selected histopathological lesions at ileal margin (ulceration, fibrosis in submucosa, lymphoplasmacytic infiltrate in subserosa). No lesion: patients with normal ileal margin. Crohn's disease (CD) lesions but not transmural: patients with ileal margin affected but without CD transmural lesions defined by the presence of mucosal ulceration or cryptitis, submucosal fibrosis, and lymphoplasmacytic infiltrate of the subserosa. CD transmural lesions: patients with these 3 lesions on ileal margin. (*A*) Whole cohort. (*B*) Patients with no postoperative biotherapy.

(recurrence rate: 58.3% vs. 45.7%; OR, 1.66; 95% CI, 0.91–3.07; $P = .09$) (Table 2). Submucosal and myenteric plexitis were not associated with endoscopic recurrence.

Based on this univariate analysis, we defined a posteriori the item "CD transmural lesions" by the presence of mucosal ulceration or cryptitis, submucosal fibrosis, and lymphoplasmacytic infiltrate of the subserosa. This histological feature was observed in 28 patients (13.6%) of the 206 samples in which these 3 lesions could be assessed. These 28 patients had a significant increased risk of endoscopic recurrence when compared with those without this histological feature ($P = .005$) (Figure 2*A*). Severe recurrence (ie, score i3 or i4) was also more frequent in patients with CD transmural lesions on margin ($P = .03$) (Figure 2*A*). The sensitivity and specificity of the presence of CD transmural lesions for the risk of endoscopic recurrence was 20% and 93%, respectively, with an accuracy of 63%. Consequently, the positive and negative predictive values were of 75% and 53%, respectively.

Only 8 of the 28 patients who had CD transmural lesions received postoperative anti-TNF. Only 2 were i0 and 6 had a recurrence ($\geq$ i2). In patients with no postoperative biotherapy, CD transmural lesions were also associated with endoscopic recurrence ($P = .03$) (Figure 2*B*).

We integrated the selected lesions indicative of CD transmural lesions in a multivariate analysis with the clinical parameters associated with an increased risk of endoscopic recurrence in our cohort (ie, male sex, previous intestinal surgery, active smoker at surgery, and postoperative preventive anti-TNF treatment).[7] CD transmural lesions remained independently associated with endoscopic recurrence (OR, 3.83; 95%CI, 1.47–11.05; $P = .008$) (Table 3).

Correlation With Long-Term Clinical Outcome

In univariate analysis, clinical parameters significantly associated with long-term clinical recurrence were: active smoking at time of surgery ($P = .007$), previous intestinal surgery ($P = .012$), and presence of extraintestinal manifestations at surgery ($P = .049$). CD transmural lesions at the ileal margin were also associated with a significantly poorer outcome ($P = .022$) (Figure 3).

In multivariate analysis, the 4 factors identified in univariate analysis, including CD transmural lesions at ileal margin, remained independently associated with clinical recurrence (Table 3).

Table 3. Correlation Among Clinical Factors, CD Transmural Lesions of the Ileal Margin and Both Endoscopic and Clinical Recurrences

	Correlation With Endoscopic Recurrence (Multivariate Analysis)			Correlation With Clinical Long Term Recurrence (Multivariate Analysis)		
	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*
Age	0.98	0.95–1.01	.082	1.01	0.99–1.03	.320
Male	2.31	1.26–4.32	.008	1.39	0.85–2.28	.189
Previous surgery	5.46	2.22–14.57	<.001	1.86	1.01–3.27	.046
Active smokers	2.78	1.47–5.39	<.001	1.69	1.15–2.95	.011
Postoperative anti-TNF	0.42	0.21–0.84	.016	NA	NA	NA
Extradigestive manifestations	NA	NA	NA	2.11	1.19–3.63	.010
CD transmural lesions	3.83	1.47–11.05	.008	2.04	1.09–3.99	.026

Multivariate analysis. Significance was reached if $P < .05$.
CD, Crohn's disease; CI, confidence interval; NA, •••; TNF, tumor necrosis factor. Q10

Discussion

This prospective study is, to our knowledge, the largest analyzing the impact of macroscopic and microscopic CD involvement of the ileal resection margin on endoscopic and clinical outcomes after ileocaecal resection. CD transmural lesions at ileal margin were strongly correlated with both early postoperative endoscopic recurrence and long-term clinical outcome independent of other risk factors. Conversely, we did not find any correlation between reported macroscopic ulceration noticed on the ileal margin and recurrence.

About one-third of our patients had a microscopically normal ileal margin while the macroscopic evaluation didn't notice mucosal ulcerations in 80% of patients. This difference highlights the need for a complete microscopic evaluation of the bowel wall to fully assess tissue injury.

A recent literature review emphasized the crucial need of new studies assessing the involvement of both macroscopic and microscopic lesions in CD recurrence after surgery.[24] Two recent publications found an association between microscopic lesions at ileal margins and recurrence in both adult and pediatric patients.[22,25] However, no detail on the type of lesions and their location in the intestinal wall were provided. Herein, 2 experts pathologists assessed each CD histological lesions, including plexitis, using a reproducible grid. Thus, in contrast to recent literature on the subject,[15–21] we considered all CD-related lesions potentially found in the whole bowel wall through a complete

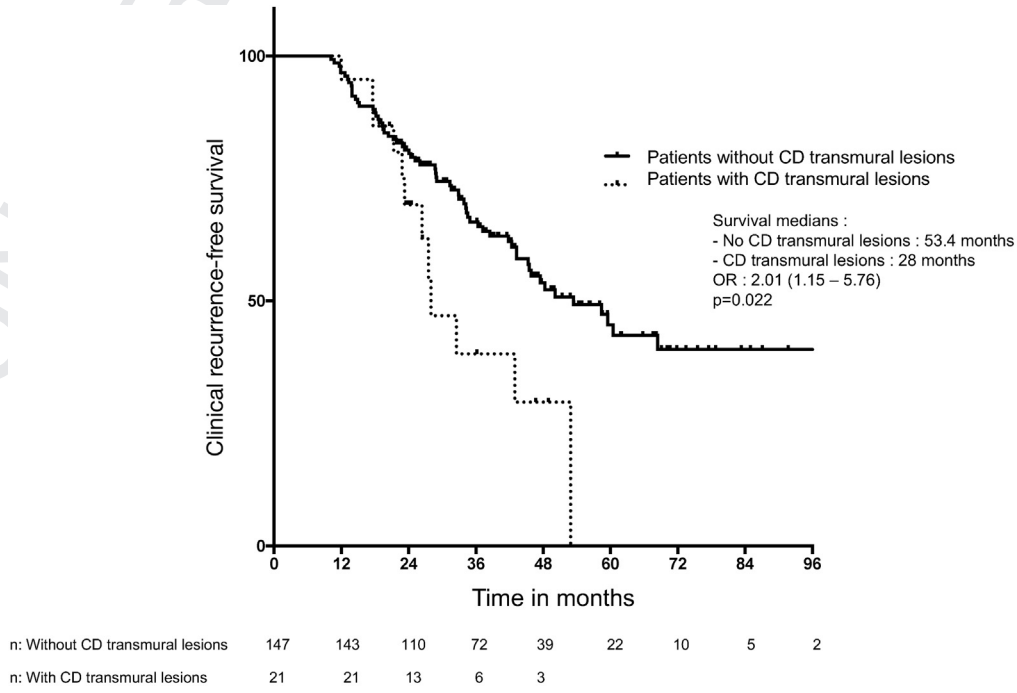

Figure 3. Clinical recurrence-free survival depending on selected histopathological lesions at ileal margins. Survival curves were built using Kaplan-Meier method and crude log rank tests were calculated. Significance was reached if $P < .05$. CD, Crohn's disease.

and reproducible analysis rather than focusing on plexitis alone. In our cohort, plexitis, whatever its severity, was not associated with recurrence. Interestingly, most of the patients with grade 3 MP had significant lesions in other layers, while few patients (n = 6) had plexitis only.

We hypothesized that some histological lesions could be more relevant than others and selected them from our statistical analysis. In univariate analysis, 2 lesions were significantly associated with endoscopic recurrence—fibrosis of the submucosa and lymphoplasmacytic infiltrate of the subserosa—reflecting sequelae of previous CD flares or persistent chronic inflammation. Moreover, mucosal lesions such as ulceration or cryptitis were also associated with a trend toward recurrence. These 3 lesions had a similar impact on postoperative recurrence (ORs for ulceration or cryptitis, fibrosis of the submucosa, and lymphoplasmacytic infiltrate of the subserosa were 1.66 [95% CI, 0.91–3.07], 1.80 [95% CI, 1.01–3.21], and 1.89 [95% CI, 1.01–3.51], respectively). Thus, we used the combination of these 3 CD-related lesions, each affecting 1 layer of the bowel wall, to define intense and active CD transmural involvement of the ileal margin, which was strongly associated with recurrence. This finding highlights that, rather than isolated microscopic lesions, deep CD-related tissue injury of the ileal margin could influence recurrence risk. These patients could beneficiate from an immediate postoperative treatment.

No association was found between macroscopic examination of the opened surgical specimen (length of ileal resection and distance from margin to first macroscopic ulceration) and rates of clinical or endoscopic recurrence. These data are in alignment with a previous study using frozen section examination of resection margins during surgery to achieve microscopical clearance of CD lesions.[26] This approach did not improve short- and long-term patient's clinical outcomes. Our study also does not support extensive resections. We believe that surgeons should still perform limited resections.

CD is characterized by discontinuous patchy or extensive transmural inflammation with skip lesions. Recurrence remains frequent despite normal resection margins. Indeed, 46% of patients with normal ileal margin experienced endoscopic recurrence, which was even severe in 10% (i3 or i4). Histological evaluation of the margin has therefore a low sensitivity for prediction of postoperative recurrence.

Our study has several limitations. First, the 2 pathologists analyzed jointly the histological slides and we did not assess interobserver variability. Second, there was no endoscopic central reading, which could have led to misclassifications between i1 and i2. However, 2 physicians checked the colonoscopies report blinded of treatment and patients outcomes, which could have limited this potential bias. Third, long-term clinical outcomes were collected retrospectively. Moreover, in this large prospective cohort, some patients were lost to follow-up. However, no difference was found between the 168 patients with long-term data and the whole cohort. Finally, we did not assess the colonic margin, in which Inflammation could potentially impact postoperative recurrence risk in a similar way.

In conclusion, we show in this prospective multicenter cohort study that CD transmural histological lesions at the ileal margin of ileocecal resection specimens in CD were associated with an increased risk of endoscopic and clinical recurrence. Pathologists should systematically detail in their reports the analysis of the ileal margin of resection notifying the presence of CD transmural lesions (ulceration or erosion, fibrosis, and lymphoplasmacytic infiltrate). A systematic postoperative treatment could be proposed to patients with transmural histological lesions, even in the absence of other risk factors. The better postoperative treatment to initiate to these patients remains to be determined (NCT03458195).

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2019.04.045.

References

1. Binder V, Hendriksen C, Kreiner S. Prognosis in Crohn's disease–based on results from a regional patient group from the county of Copenhagen. Gut 1985;26:146–150.
2. Cosnes J, Nion-Larmurier I, Beaugerie L, et al. Impact of the increasing use of immunosuppressants in Crohn's disease on the need for intestinal surgery. Gut 2005;54:237–241.
3. Peyrin-Biroulet L, Harmsen WS, Tremaine WJ, et al. Surgery in a population-based cohort of Crohn's disease from Olmsted County, Minnesota (1970–2004). Am J Gastroenterol 2012; 107:1693–1701.
4. Rutgeerts P, Geboes K, Vantrappen G, et al. Natural history of recurrent Crohn's disease at the ileocolonic anastomosis after curative surgery. Gut 1984;25:665–672.
5. Gionchetti P, Dignass A, Danese S, et al. 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: part 2: surgical management and special situations. J Crohn's Colitis 2017;11:135–149.
6. Buisson A, Chevaux J-B, Allen PB, et al. Review article: the natural history of postoperative Crohn's disease recurrence. Aliment Pharmacol Ther 2012;35:625–633.
7. Auzolle C, Nancey S, Tran-Minh ML, et al. Male gender, active smoking and previous intestinal resection are risk factors of postoperative endoscopic recurrence in Crohn's disease: results from a prospective cohort study. Aliment Pharmacol Ther 2018;48:924–932.
8. Singh S. Editorial: predicting recurrence of Crohn's disease after surgical resection-close to a crystal ball. Aliment Pharmacol Ther 2018;48:1156–1157.
9. Fazio VW, Marchetti F, Church M, et al. Effect of resection margins on the recurrence of Crohn's disease in the small

bowel. A randomized controlled trial. Ann Surg 1996;224: 563–713.
10. Pennington L, Hamilton SR, Bayless TM, et al. Surgical management of Crohn's disease. Influence of disease at margin of resection. Ann Surg 1980;192:311–318.
11. Cooper JC, Williams NS. The influence of microscopic disease at the margin of resection on recurrence rates in Crohn's disease. Ann R Coll Surg Engl 1986;68:23–26.
12. Kotanagi H, Kramer K, Fazio VW, et al. Do microscopic abnormalities at resection margins correlate with increased anastomotic recurrence in Crohn's disease? Retrospective analysis of 100 cases. Dis Colon Rectum 1991;34:909–916.
13. Heimann TM, Greenstein AJ, Lewis B, et al. Prediction of early symptomatic recurrence after intestinal resection in Crohn's disease. Ann Surg 1993;218:294–299.
14. Geboes K, Rutgeerts P, Ectors N, et al. Major histocompatibility class II expression on the small intestinal nervous system in Crohn's disease. Gastroenterology 1992;103:439–447.
15. Ferrante M, de Hertogh G, Hlavaty T, et al. The value of myenteric plexitis to predict early postoperative Crohn's disease recurrence. Gastroenterology 2006;130:1595–1606.
16. Ng SC, Lied GA, Kamm MA, et al. Predictive value and clinical significance of myenteric plexitis in Crohn's disease. Inflamm Bowel Dis 2009;15:1499–1507.
17. Sokol H, Polin V, Lavergne-Slove A, et al. Plexitis as a predictive factor of early postoperative clinical recurrence in Crohn's disease. Gut 2009;58:1218–1225.
18. Bressenot A, Chevaux J-B, Williet N, et al. Submucosal plexitis as a predictor of postoperative surgical recurrence in Crohn's disease. Inflamm Bowel Dis 2013;19:1654–1661.
19. Misteli H, Koh CE, Wang LM, et al. Myenteric plexitis at the proximal resection margin is a predictive marker for surgical recurrence of ileocaecal Crohn's disease. Colorectal Dis 2015; 17:304–310.
20. Decousus S, Boucher A-L, Joubert J, et al. Myenteric plexitis is a risk factor for endoscopic and clinical postoperative recurrence after ileocolonic resection in Crohn's disease. Dig Liver Dis 2016;48:753–758.
21. Lemmens B, de Buck van Overstraeten A, Arijs I, et al. Submucosal plexitis as a predictive factor for postoperative endoscopic recurrence in patients with Crohn's disease undergoing a resection with ileocolonic anastomosis: results from a prospective single-centre study. J Crohn's Colitis 2017; 11:212–220.
22. de Buck van Overstraeten A, Eshuis EJ, Vermeire S, et al. Short- and medium-term outcomes following primary ileocaecal resection for Crohn's disease in two specialist centres. Br J Surg 2017;104:1713–1722.
23. Rutgeerts P, Geboes K, Vantrappen G, et al. Predictability of the postoperative course of Crohn's disease. Gastroenterology 1990;99:956–963.
24. Patel KV, Darakhshan AA, Griffin N, et al. Patient optimization for surgery relating to Crohn's disease. Nat Rev Gastroenterol Hepatol 2016;13:707–719.
25. Diederen K, de Ridder L, van Rheenen P, et al. Complications and disease recurrence after primary ileocecal resection in pediatric Crohn's disease. Inflamm Bowel Dis 2017;23:272–282.
26. Hamilton SR, Reese J, Pennington L, et al. The role of resection margin frozen section in the surgical management of Crohn's disease. Surg Gynecol Obstet 1985;160:57–62.

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Conflicts of interest
These authors disclose the following: Stéphane Nancey received honoraria from MSD, Abbvie, Takeda, Janssen, HAC Pharma, Tillots, Ferring, and Novartis. Benjamin Pariente received honoraria from AbbVie, MSD, Takeda, Janssen, Bioagaran, and Ferring. Anthony Buisson received honoraria from MSD, Abbvie, Ferring, Takeda, Vifor Pharma, Sanofi-Aventis, Hospira, and Janssen. Xavier Treton received honoraria from Abbvie, MSD, Takeda, Ferring, Norgine, and Janssen. Mathurin Fumery received honoraria from AbbVie, MSD, Takeda, Janssen, Pfizer, Ferring, and Boehringer. Philippe Seksik received honoraria from Takeda, MSD, Biocodex, Ferring and Abbvie and financial support from Takeda. Jean-François Flejou declares no competing interest. Matthieu Allez received honoraria from Abbvie, MSD, Janssen, Takeda, Pfizer, Novartis, Ferring, Tillots, Celgene, and Genentech/Roche. The remaining authors disclose no conflicts.

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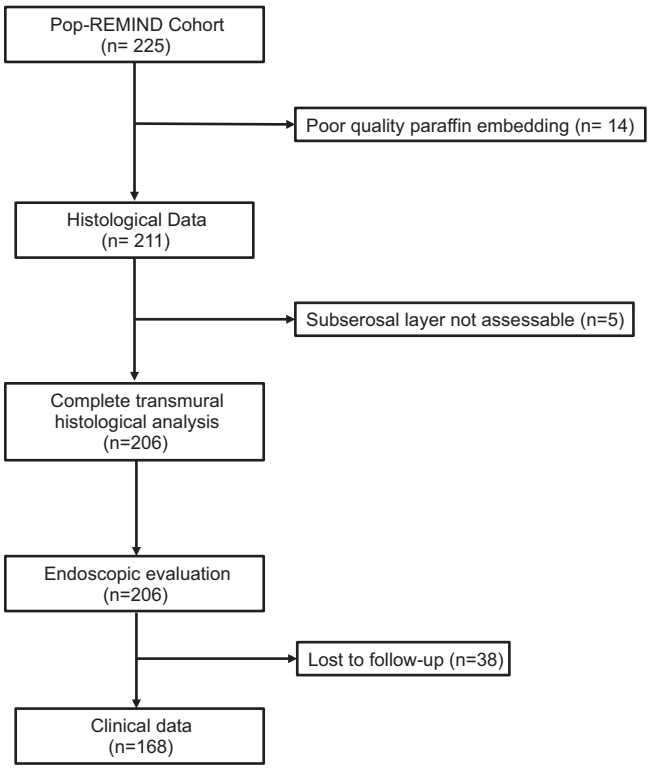
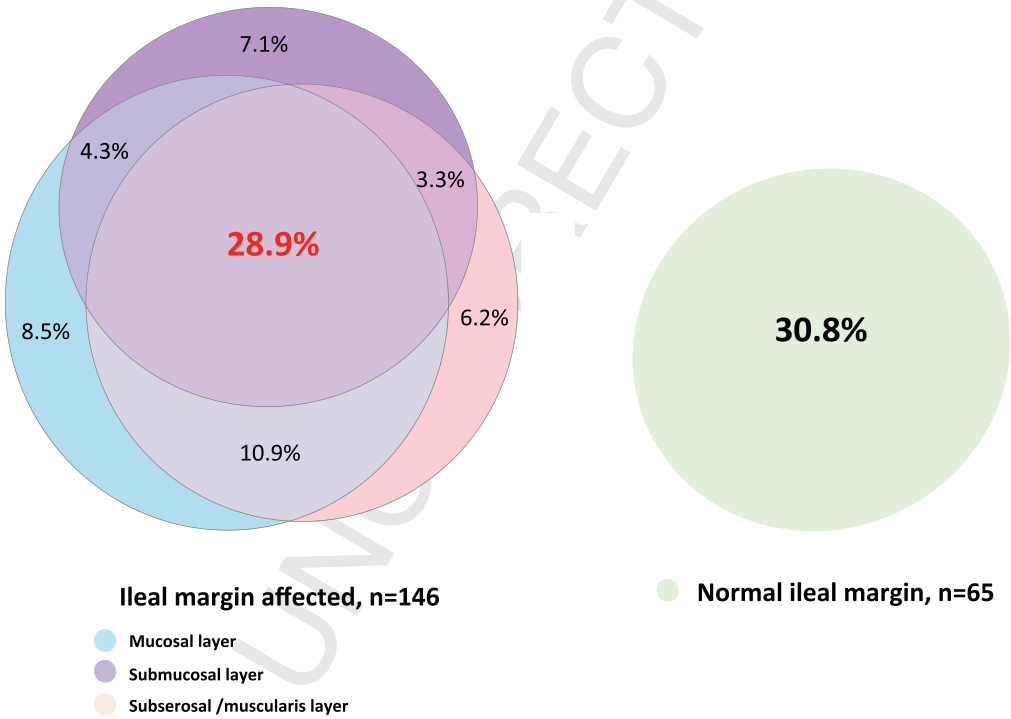
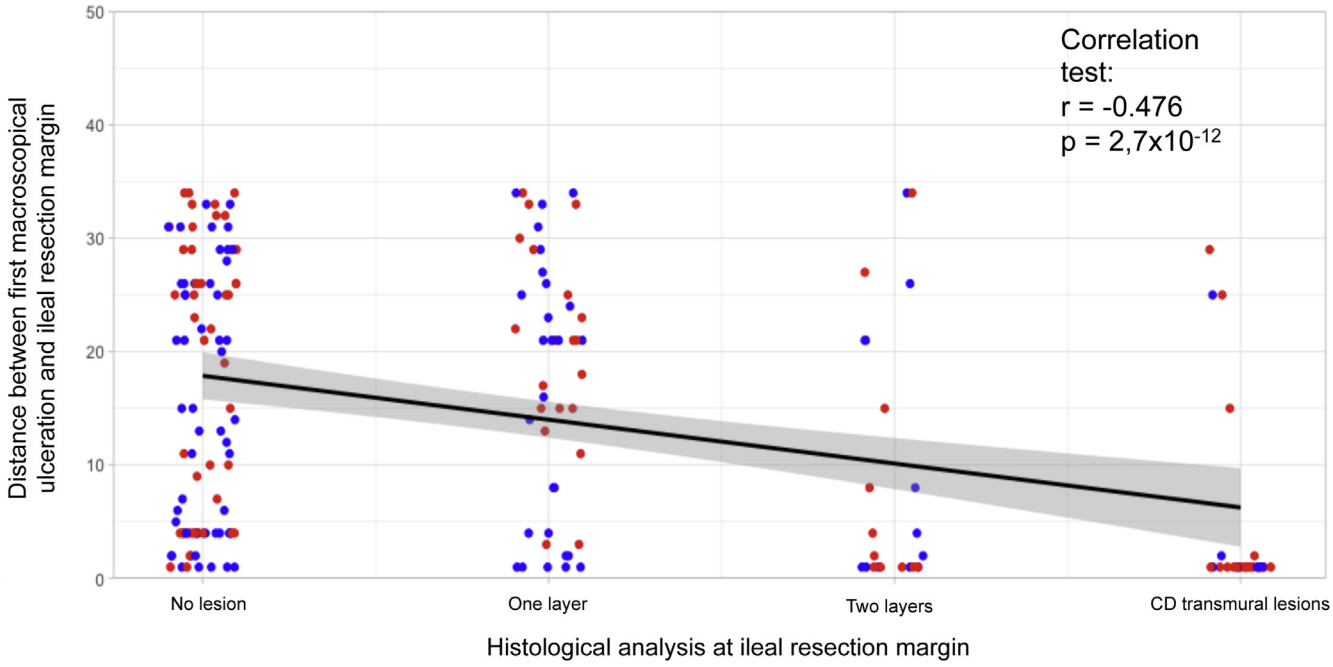


Supplementary Figure 1. Flow chart. REMIND, ●●●.



Supplementary Figure 2. Venn diagram showing distribution of the whole cohort of patients (n = 211) according to the histological data: 28.9% of patients presented an ileal margin affected with CD-related lesions in the 3 layers of the bowel wall and 30.8% had a normal ileal margin.



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Supplementary Figure 3. Distance between first macroscopical ulceration and ileal margin correlated to histological data. No lesion: patients with normal ileal margin. One layer: lesions affecting 1 layer of the intestinal wall. Two layers: lesions affecting at least 2 layers of the intestinal wall. Crohn's disease (CD) transmural lesions: presence of mucosal ulceration or cryptitis, submucosal fibrosis, and lymphoplasmocytic infiltrate in the subserosa. Red points indicate patients who experienced endoscopic recurrence and blue points for patients who did not. Correlation test performed using Spearman's rank correlation test.