



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

Nassim Hammoudi, Dominique Cazals-Hatem, Claire Auzolle, Charlotte Gardair, Marjolaine Ngollo, Hugo Bottois, Stéphane Nancey, Benjamin Pariente, Anthony Buisson, Xavier Tréton, et al.

► To cite this version:

Nassim Hammoudi, Dominique Cazals-Hatem, Claire Auzolle, Charlotte Gardair, Marjolaine Ngollo, et al.. Association Between Microscopic Lesions at Ileal Resection Margin and Recurrence After Surgery in Patients With Crohn's Disease. Clinical Gastroenterology and Hepatology, 2019, 10.1016/j.cgh.2019.04.045 . hal-02193633

HAL Id: hal-02193633

<https://hal.science/hal-02193633>

Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

Nassim Hammoudi^{*1,2}, Dominique Cazals-Hatem^{*3}, Claire Auzolle^{1,2}, Charlotte Gardair⁴, Marjolaine Ngollo¹, Hugo Bottois¹, Stéphane Nancey⁵, Benjamin Pariente⁶, Anthony Buisson⁷, Xavier Treton⁸, Mathurin Fumery⁹, Madeleine Bezault¹, Philippe Seksik¹⁰, Lionel Le Bourhis¹, REMIND study group investigators, Jean-François Flejou¹¹, Matthieu Allez^{1,2}

* Both authors contributed equally

Affiliations:

1, INSERM UMRS 1160, Université Paris Diderot, Sorbonne Paris-Cité University, Paris, France. 2, Gastroenterology Department, AP-HP, Hôpital Saint-Louis, Paris, France. 3 Pathology Department, AP-HP, Hôpital Beaujon, Clichy, France, 4, Pathology Department, AP-HP, Hôpital Saint-Louis, Paris, France. 5, Gastroenterology Department, Lyon Sud Hospital, Hospices Civils de Lyon, Lyon, France. 6 Gastroenterology Department, Hôpital Claude Huriez, University of Lille 2, Lille, France. 7 Université Clermont Auvergne, Inserm U1071, M2iSH, 3iHP, CHU Clermont-Ferrand, Service de Médecine de l'Appareil Digestif, Clermont-Ferrand, France. 8 Hôpital Beaujon, Service de Gastroentérologie, MICI et Assistance Nutritive, Clichy, France. 9 Hepatogastroenterology Department, Amiens University Hospital, Amiens, France. 10 Sorbonne Universités, Department of Gastroenterology, Saint Antoine Hospital, AP-HP, Ecole normale supérieure, PSL Research University, CNRS, INSERM, Laboratoire des Biomolécules (LBM), Paris,

France. 11 Pathology Department, AP-HP, Hôpital Saint-Antoine, Faculté de Médecine Sorbonne Université, Paris, France.

Word count:

Abstract: 274

Manuscript (including references and figure legends): 4010

Manuscript: 2951 / References: 26, 629 words / Figures and legends: 430

Pages: 26 / Tables: 3 / Figures: 3

Corresponding Author: Prof Matthieu Allez

Département de Gastroentérologie

Hôpital Saint-Louis

1 avenue Claude Vellefaux

75010 Paris, France

Email : matthieu.allez@aphp.fr

Conflict of interest statement: NH declares no competing interest. DCH declares no competing interest. CA declares no competing interest. CG declares no competing interest. MN declares no competing interest. HB declares no competing interest. SN received honoraria from MSD, Abbvie, Takeda, Janssen, HAC Pharma, Tillots, Ferring, and Novartis. BP received honoraria from AbbVie, MSD, Takeda, Janssen, Bioagaran, and Ferring. AB received honoraria from MSD, Abbvie, Ferring, Takeda, Vifor Pharma, Sanofi-Aventis, Hospira, and Janssen. XT received honoraria from Abbvie, MSD, Takeda, Ferring, Norgine, and Janssen. MF received honoraria from AbbVie, MSD, Takeda, Janssen, Pfizer, Ferring, and Boehringer. MB declares no

competing interest. LLB declares no competing interest. PS received honoraria from Takeda, MSD, Biocodex, Ferring and Abbvie and financial support from Takeda. JFF declares no competing interest. MA received honoraria from Abbvie, MSD, Janssen, Takeda, Pfizer, Novartis, Ferring, Tillots, Celgene, and Genentech/Roche.

Guarantor of the article: Matthieu Allez

Author contributions: NH: histological analysis, interpretation of data and drafting of the manuscript; DCH: histological analysis, design, supervision of histological analysis and critical review of the manuscript; CA: statistical analysis and critical review of the manuscript; CG: histological analysis and critical review of the manuscript; HB, MN, SN, BP, AB, XT, MF, PS, LLB: data collection and critical review of the manuscript; MB: management of the study; JFF: design of histological analysis, critical review of the manuscript; MA: conception, design and supervision of the study, analysis of data, drafting and critical review of the manuscript. All authors reviewed and approved the final version of the manuscript.

ABSTRACT

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 lymphoplasmocytic 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.

91

92 **KEYWORDS**

93

94 REMIND trial

95

96 Outcome

97

98 Prognostic Factor

99

100 IBD Surgery

101

102

103 **ABBREVIATIONS**

104

105 CD: Crohn's Disease

106

107 HES: Hematosin Eosin Saffron

108

109 IQR: Inter Quartile Range

110

111 MP: Myenteric Plexitis

112

113 OR: Odds Ratio

INTRODUCTION

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 post-operative recurrence occurs in the majority of patients. Early post-operative endoscopic recurrence, which is mainly observed at the anastomosis or in the neo-terminal ileum, precedes clinical recurrence and its severity predicts the long-term clinical outcome (4). Several risk factors have been reported to increase post-operative recurrence, including active smoking, penetrating disease, history of perianal disease, previous intestinal surgery and male gender (5-7). There is still a need for factors that can accurately risk-stratify patients (8).

Macroscopically involved ileal margin could increase post-operative recurrence risk but this assumption was pushed aside in 1996 by Fazio et al with the results of a randomized controlled trial showing no benefit of extended resection (clearance of 12 cm versus 2 cm of normal ileal margin) (9). Retrospective studies showed conflicting results regarding the impact of CD microscopic lesions on the ileal margin (mainly granuloma or active inflammation) on post-operative 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 two 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).

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

MATERIALS AND METHODS

Post-operative 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 post-operative endoscopic recurrence (7). Patients were included between September 2010 and December 2016. Inclusion criteria were: age >18 years, ileal or ileocolonic CD and indication of CD-related intestinal surgery (ileocolonic resection). A post-operative treatment was recommended according to a pre-established 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 AFSAPS (IDRCB: 2009-A00205-52), 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 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 post-operative endoscopy. They included demographical data (gender, 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, extra-digestive manifestations, treatment history including previous exposure to immunosuppressants (azathioprine, 6-MP or methotrexate) and anti-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 neo-terminal ileum. The physician who performed the colonoscopy evaluated the Rutgeerts score for each patient. Two physicians (MA and CA) checked the colonoscopy's report blinded to histological analysis and post-operative 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 and/or CT 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- μ m section and stained with hematein eosin saffron (HES). Each section was jointly analyzed by two expert pathologists (DCH and CG) blinded to the baseline detailed clinical data and to the post-operative outcomes of the patients. An analysis grid was built to assess CD-related lesions affecting each intestinal wall layer (mucosa, submucosa, and subserosa/muscularis).

Each of these lesions was considered as binary variable except for plexitis, for which three 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

Post-operative endoscopic recurrence was evaluated 6 months after surgery. Endoscopic recurrence was defined as a Rutgeerts score \geq i2.

Clinical recurrence was defined by: (i) CD-related clinical manifestations confirmed either by endoscopy (Rutgeerts score \geq i2 or severe colonic lesions in one 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), (ii) CD-related complications

(intra-abdominal abscess or occlusive manifestation) or (iii) CD-related subsequent surgery.

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

Statistical analyses

Descriptive statistics were calculated for all quantitative variables as medians with their respective interquartile ranges (IQR). Correlation between histology and recurrence, for each item of the analysis grid, was evaluated by both univariate and multivariate analyses. Chi-2 and Logistic regression were respectively used for binary variables. For continuous data (lengths in centimeters), 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 (gender and age) and variables significantly associated with clinical or surgical recurrence in univariate analyses was built.

All statistical analyses were two-tailed and a p value of less than 0.05 was considered as statistically significant. To perform these analyses, R software ® (3.2.2 version) and PrismGraphPad ® 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 years (IQR: 30.7-47.5) and median disease duration was 6.3 years (IQR: 1.8-11.9). Ninety-two (44%) patients were male, 67 (32%) active smokers and 38 (18%) had a previous ileocaecal resection in their past medical history (**Table 1**). Post-operative treatment was given to 102 (48%) patients, including an anti-TNF treatment in 52 (25%) patients.

Post-operative endoscopy was performed in all patients with a median time between resection and colonoscopy of 6.8 months. One hundred and four patients (49%) had an endoscopic recurrence (i.e. Rutgeerts score \geq i2) and 43 (20%) patients had severe lesions (i.e. 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, post-operative management and endoscopic recurrence rates of these 168 patients did not differ significantly from the whole cohort.

Median follow-up was 3.8 years (IQR: 2.55-5.41). 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).

268

269 ***Histopathological analysis of ileal margin***

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

275 Microscopically, grade 1 MP was found on the ileal margin of the 10 control
276 patients revealing CD-unrelated lesion (discarded from the final grid). Among the 211
277 patients studied, 65 (30.8%) had a normal ileal margin and 146 (69.2%) had at least
278 one CD lesion (**Table 2**). The most affected layer of the bowel wall was the mucosa
279 (111 patients (52.6%)). Sixty patients (28.4%) had mucosal ulceration or cryptitis
280 indicative of CD activity. The submucosal layer was affected in 104 (49.3%) patients,
281 16 (7.8%) with neutrophils infiltrate (all associated with active mucosal lesion). The
282 most frequent submucosal lesions were lymphoplasmocytic infiltrate (40%) and
283 fibrosis (33.8%). All patients with submucosal plexitis (38.6%) had concomitant
284 lymphoplasmocytic infiltrate. The subserosa/muscularis (plexitis included) were
285 affected in 92 (44.7%) patients. The subserosa was affected in 64 (31.1%) patients
286 (**Supplementary Figure 2**).

287 Patients with at least one lesion of each layer frequently presented grade 2 or
288 3 MP (respectively 21 (55%) patients and 12 (33%)). Interestingly, plexitis (either
289 submucosal or myenteric) was the only lesion found in 6 patients (3%).

290 The presence of microscopic lesions was inversely correlated with the
291 distance between ileal margin and macroscopic ulceration: patients with transmural
292 lesions had a shorter disease-free margin when compared to patients with 2, 1 or

none of the three histological features defining transmural impairment (Spearman's rank correlation test: $r=-0.476$; $p<0.001$) (**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 ($p=0.96$, $p=0.31$ and $p=0.43$ respectively).

In univariate analysis, two histological lesions were significantly associated with endoscopic recurrence: submucosal fibrosis (recurrence rate: 59.1% vs. 44.6%, OR=1.80, 95%CI [1.01-3.21], $p=0.04$) and lymphoplasmocytic infiltrate of the subserosa (recurrence rate: 60.3% vs. 44.6%, OR=1.89, 95%CI [1.01-3.51], $p=0.04$) (**Table 2**). A trend towards recurrence was also found in patients displaying mucosal ulceration or cryptitis indicative of active CD (recurrence rate: 58.3% vs. 45.7%, OR=1.66, 95%CI [0.91-3.07], $p=0.09$) (**Table 2**). Submucosal or 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 lymphoplasmocytic infiltrate of the subserosa. This histological feature was observed in 28 patients (13.6%) out of the 206 samples in which these three lesions could be assessed. These 28 patients had a significant increased risk of endoscopic recurrence when compared to those without this histological feature ($p=0.005$) (**Figure 2a**). Severe recurrence (i.e. score i3 or i4) was also more frequent in patients with CD transmural lesions on margin ($p=0.03$) (**Figure 2a**). 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 post-operative anti-TNF. Only 2 were i0 and 6 had a recurrence (\geq i2). In patients with no post-operative biotherapy, CD transmural lesions were also associated with endoscopic recurrence ($p=0.03$, **figure 2b**).

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 (i.e. male gender, previous intestinal surgery, active smoker at surgery and post-operative preventive anti-TNF treatment (7)). CD transmural lesions remained independently associated with endoscopic recurrence (OR: 3.83, 95%CI [1.47-11.05], $p=0.008$) (**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 ($p=0.007$), previous intestinal surgery ($p=0.012$), and presence of extra-intestinal manifestations at surgery ($p=0.049$). CD transmural lesions at the ileal margin were also associated with a significantly poorer outcome ($p=0.022$) (**Figure 3**).

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

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 post-operative endoscopic recurrence and long-term clinical outcome independent of other risk factors. Conversely, we didn't 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, two 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 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, two lesions were significantly associated with endoscopic recurrence - fibrosis of the submucosa and lymphoplasmocytic 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 towards recurrence. These 3 lesions had a similar impact on post-operative recurrence (odds ratios for ulceration/cryptitis, fibrosis of the submucosa and lymphoplasmocytic infiltrate of the subserosa were 1.66 [0.91-3.07], 1.80 [1.01-3.21] and 1.89 [1.01-3.51] respectively). Thus, we used the combination of these 3 CD-related lesions, each affecting one 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 benefit from an immediate post-operative 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 post-operative recurrence.

Our study has several limitations. First, the two pathologists analyzed jointly the histological slides and we did not assess inter-observer variability. Second, there was no endoscopic central reading, which could have led to misclassifications between i1 and i2. However, two 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 post-operative 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 ileocaecal 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/erosion, fibrosis and lymphoplasmocytic infiltrate). A systematic post-operative treatment could be proposed to patients with transmural histological lesions, even in absence of other risk factors. The better post-operative treatment to initiate to these patients remains to be determined; ClinicalTrials.gov number, NCT03458195.

415 REMIND STUDY GROUP INVESTIGATORS
416

417 Saint-Louis, Paris: Pierre Cattán, Mircea Chirica, Nicolas Munoz-Bongrand, Hélène
418 Corte, Nathan Beaupel, Jonathan Catry, Jean-Marc Gornet, Clotilde Baudry, Nelson
419 Lourenco, Mariane Maillet, My-Linh Tran-Minh, Victor Chardiny, Céline Grand, Brice
420 Gergaud, Joelle Bonnet, Leila Chedouba, Andrée Nisard; Saint-Antoine, Paris:
421 Laurent Beaugerie, Harry Sokol, Anne Bourrier, Isabelle Nion-Larmurier, Julien
422 Kirchgesner, Elodie Quevrain, Loic Brot, Najim Chafai, Jeremie H. Lefevre,
423 Emmanuel Tiret, Magali Svrcek; Beaujon, Clichy: Nathalie Guedj, Yves Panis, Leon
424 Magiorri, Marianne Ferron, Yoram Bouhnik, Olivier Corcos, Carmen Stefanescu;
425 Lariboisière, Paris: Philippe Marteau, Xavier Dray, Ulrika Chaput, Rachid Kaci;
426 Clermont-Ferrand: Anne Dubois, Gilles Bommelaer, Marion Goutte, Nicolas Barnich,
427 Dilek Coban, Catherine Godfraind, Juliette Joubert Zakeyh ; Lille: Pierre
428 Desreumaux, Maria Nachury, Coralie Sommeville, Florence Renaud; Amiens: Jean-
429 Louis Dupas, Julien Loreau, Franck Brazier, Denis Chatelain, Christophe Attencourt,
430 Charles Sabbagh, Martine Leconte; Lyon: Gilles Boschetti, Bernard Flourié, Yves
431 François, Eddy Cotte, Anne-Laure Charlois, Peggy Falgon, Helena Hadjisavvas,
432 Driffa Moussata, Marion Chauvenet, Sarah Boyer, Alexandra Traverse-Glehen; Nice:
433 Xavier Hebuterne, Jérôme Filippi, Paul Hofmann, Amine Rahili, Stéphanie
434 Patouraux. Statistiques, PARCC Paris: Xavier Jouven

435

436 ACKNOWLEDGMENTS

437 We thank the Association Francois Aupetit, Helmsley Charitable Trust, MSD France,
438 and La Fondation pour la Recherche Médicale (DEA20170637874) for their funding
439 grants.

440 We also acknowledge Nathalie Colnot for her technical support.

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(2):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(2):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(11):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(6):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(2):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(6):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(10):1156-1157
- 8-Singh S. Editorial: predicting recurrence of Crohn's disease after surgical resection-close to a crystal ball.*Aliment Pharmacol Ther*.2018;48(10):1156–7.

466 **9**-Fazio VW, Marchetti F, Church M, et al. Effect of resection margins on the
 467 recurrence of Crohn's disease in the small bowel. A randomized controlled trial.*Ann*
 468 *Surg.*1996;224(4):563-71-3.

469 **10**-Pennington L, Hamilton SR, Bayless TM, et al. Surgical management of Crohn's
 470 disease. Influence of disease at margin of resection.*Ann Surg.*1980;192(3):311-318.

471 **11**-Cooper JC, Williams NS. The influence of microscopic disease at the margin of
 472 resection on recurrence rates in Crohn's disease.*Ann R Coll Surg*
 473 *Engl.*1986;68(1):23-26.

474 **12**-Kotanagi H, Kramer K, Fazio VW, et al. Do microscopic abnormalities at resection
 475 margins correlate with increased anastomotic recurrence in Crohn's disease?
 476 Retrospective analysis of 100 cases.*Dis Colon Rectum.*1991;34(10):909-916.

477 **13**-Heimann TM, Greenstein AJ, Lewis B, et al. Prediction of early symptomatic
 478 recurrence after intestinal resection in Crohn's disease.*Ann Surg.*1993;218(3):294-8-
 479 9.

480 **14**-Geboes K, Rutgeerts P, Ectors N, et al. Major histocompatibility class II
 481 expression on the small intestinal nervous system in Crohn's
 482 disease.*Gastroenterology.*1992;103:439-447.

483 **15**-Ferrante M, de Hertogh G, Hlavaty T, et al. The Value of Myenteric Plexitis to
 484 Predict Early Postoperative Crohn's Disease
 485 Recurrence.*Gastroenterology.*2006;130(6):1595-1606.

486 **16**-Ng SC, Lied GA, Kamm MA, et al. Predictive value and clinical significance of
 487 myenteric plexitis in Crohn's disease.*Inflamm Bowel Dis.*2009;15(10):1499-1507.

488 **17**-Sokol H, Polin V, Lavergne-Slove A, et al. Plexitis as a predictive factor of early
 489 postoperative clinical recurrence in Crohn's disease.*Gut.*2009;58(9):1218-1225.

490 **18**-Bressenot A, Chevaux J-B, Williet N, et al. Submucosal Plexitis as a Predictor of
 491 Postoperative Surgical Recurrence in Crohn's Disease.*Inflamm Bowel*
 492 *Dis.*2013;19(8):1654-1661.

493 **19**-Misteli H, Koh CE, Wang LM, et al. Myenteric plexitis at the proximal resection
 494 margin is a predictive marker for surgical recurrence of ileocaecal Crohn's
 495 disease.*Colorectal Dis.*2015;17(4):304-310.

496 **20**-Decousus S, Boucher A-L, Joubert J, et al. Myenteric plexitis is a risk factor for
 497 endoscopic and clinical postoperative recurrence after ileocolonic resection in
 498 Crohn's disease.*Dig Liver Dis.*2016;48(7):753-758.

499 **21**-Lemmens B, de Buck van Overstraeten A, Arijis I, et al. Submucosal Plexitis as a
 500 Predictive Factor for Postoperative Endoscopic Recurrence in Patients with Crohn's
 501 Disease Undergoing a Resection with Ileocolonic Anastomosis: Results from a
 502 Prospective Single-centre Study.*J Crohn's Colitis.*2017;11(2):212-220.

503 **22**-de Buck van Overstraeten A, Eshuis EJ, Vermeire S, et al. Short- and medium-
 504 term outcomes following primary ileocaecal resection for Crohn's disease in two
 505 specialist centres.*Br J Surg.*2017;104(12):1713-1722.

506 **23**-Rutgeerts P, Geboes K, Vantrappen G, et al. Predictability of the postoperative
 507 course of Crohn's disease.*Gastroenterology.*1990;99(4):956-963.

508 **24**-Patel K V., Darakhshan AA, Griffin N, et al. Patient optimization for surgery
 509 relating to Crohn's disease.*Nat Rev Gastroenterol Hepatol.*2016;13(12):707-719.

510 **25**-Diederer K, de Ridder L, van Rheeën P, et al. Complications and Disease
 511 Recurrence After Primary Ileocecal Resection in Pediatric Crohn's Disease.*Inflamm*
 512 *Bowel Dis.*2017;23(2):272-282.

513 **26**-Hamilton SR, Reese J, Pennington L, et al. The role of resection margin frozen
514 section in the surgical management of Crohn's disease.*Surg Gynecol*
515 *Obstet.*1985;160(1):57-62.

516

517

518

519

TABLE LEGENDS

Table 1: Patients' characteristics at time of surgery

Table 2: Pathological data after examination of the ileal margin of the 211 patients and correlation of each histological lesion with endoscopic recurrence.

The margin is "affected" if at least one histological lesion is observed in one of the bowel wall layer (as defined below). Margin macroscopically affected is defined as mucosal ulceration observed by the pathologist on the ileal margin when opening the surgical specimen

Granuloma=nest of at least 5 epithelioid cells in the wall; Eosinophilic infiltrate=nest of at least 50 eosinophils in the lamina propria at x400 magnification; Villous atrophy=extensive subtotal / total villous atrophy

Pyloric Metaplasia=metaplastic glands of the ileal mucosa

Ulceration/Cryptitis=acute inflammation (neutrophils) in epithelium or crypt

Neutrophilic infiltrate=nest of at least 50 neutrophils in the submucosae at x400 magnification; Lymphoplasmocytic infiltrate=nest of at least 50 lymphocytes or plasma cells at x400 magnification; Plexitis=inflammatory cells around or inside

submucosal or myenteric plexus as defined previously (14) (Grade 1 Myenteric

Plexitis (MP: at least 1 but less than 4 immune cells within or contiguous to the

plexus, Grade 2 MP: at least 4 but less than 10 immune cells and Grade 3 MP: at least 10 immune cells);

Nervous hyperplasia=enlarged nervous bundles visible at x100 magnification;

Fibrosis=significant collagen deposit in intestinal layer visible at x100 magnification.

Table 3: Correlation between clinical factors, CD transmural lesions of the ileal margin and both endoscopic and clinical recurrences.

Multivariate analysis. Significance was reached if $p < 0.05$.

FIGURES LEGENDS

Figure 1: Pathological analysis of the whole ileal margin on surgical specimen and on section stained by hematein-eosin-saffron. (A)-Macroscopic view of an opened ileocaecal 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 CD-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.

Figure 2: Endoscopic recurrence rates depending on selected histopathological lesions at ileal margin (ulceration, fibrosis in submucosa, lymphoplasmocytic infiltrate in subserosa).

No lesion: patients with normal ileal margin. 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 lymphoplasmocytic infiltrate of the subserosa. CD transmural lesions: patients with these three lesions on ileal margin. A: Whole cohort. B: Patients with no post-operative biotherapy.

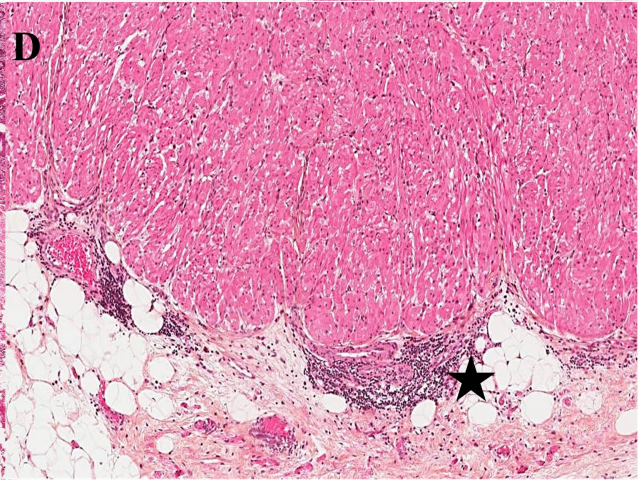
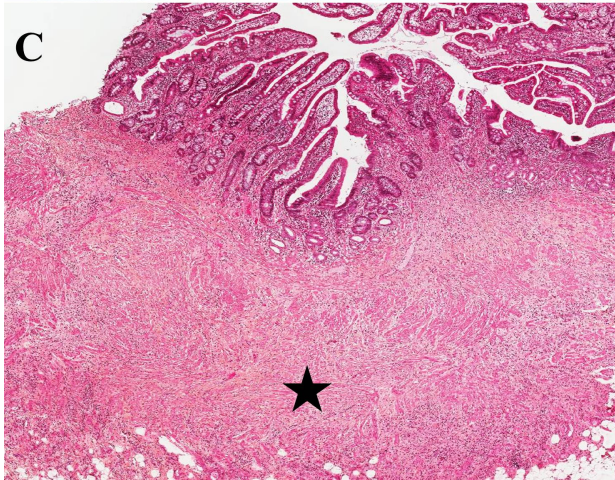
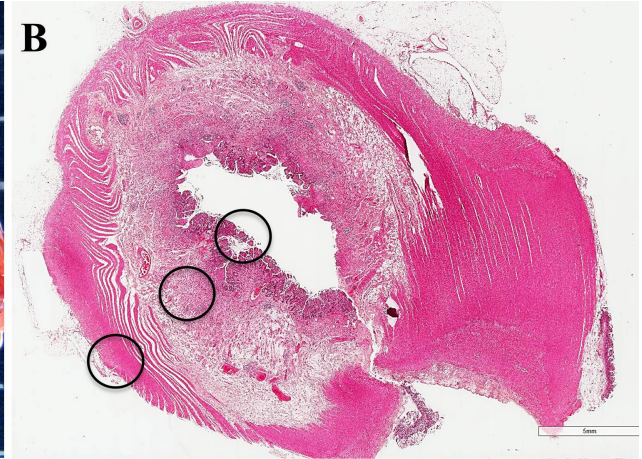
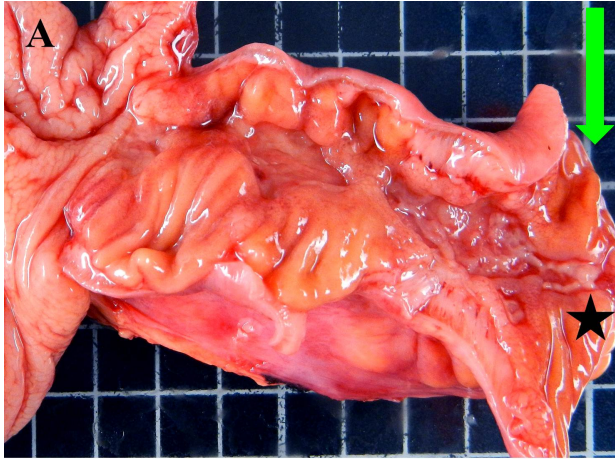
Figure 3: Clinical recurrence-free survival depending on selected histopathological lesions at ileal margins. Survival curves were built using Kaplan-Meier method and crude log rank tests were calculated. Significance was reached if $p < 0.05$.

Patients' characteristics at time of surgery	Patients n=211 (%)
Men	92 (44%)
Median age (y, IQR)	36.3 (30.7 – 47.5)
Age at Crohn's Disease diagnosis (Montreal classification) <ul style="list-style-type: none"> - ≤16 years (A1) - 17 - 40 years (A2) - >40 years (A3) 	19 (9%) 170 (81%) 22 (10%)
Median disease duration (y, IQR)	6.3 (1.8 – 11.9)
Median time between resection and colonoscopy (mo, IQR)	6.8 (6.1 – 8.4)
Smoking <ul style="list-style-type: none"> - Active smoker - Smoking cessation at surgery - Non smoker 	67 (32%) 13 (6%) 131 (62%)
Previous intestinal resection - <10 years	38 (18%) 14 (7%)
Number of previous resection(s) <ul style="list-style-type: none"> - 0 - 1 - 2 - 3 	173 (82%) 27 (13%) 7 (3%) 4 (2%)
Surgical indication <ul style="list-style-type: none"> - Stricturing complication - Penetrating complication - Failure of drug therapy 	115 (55%) 83 (39%) 13 (6%)
Disease location (Montreal classification) <ul style="list-style-type: none"> - Ileal (L1) - Ileocolonic (L3) - Anoperineal lesion 	132 (63%) 79 (37%) 50 (24%)
Extra-digestive symptoms <ul style="list-style-type: none"> - Joint manifestations - Skin manifestations - Eye manifestations 	40 (19%) 12 (6%) 4 (2%)
Previous exposure to anti-TNF therapy Anti-TNF therapy within 3 months before surgery	134 (64%) 107 (51%)
Previous exposure to thiopurines Thiopurines within 3 months before surgery	145 (69%) 60 (28%)

MICROSCOPIC DATA	Histological lesions	Prevalence (affected/analyzed)	Endoscopic recurrence (%)	<i>p</i>
Normal margin		65/211 (30.8%)	46.2	0.54
Margin affected		146/211 (69.2%)	50.7	0.54
<i>Mucosal layer</i>		111/211 (52.6%)	51.4	0.58
	Granuloma	18/211 (8.5%)	55.6	0.58
	Eosinophilic Infiltrate	68/182 (37.4%)	48.5	0.88
	Ulceration/Cryptitis	60/211 (28.4%)	58.3	0.09
	Villous Atrophy	20/211 (9.5%)	55.0	0.59
	Pyloric Metaplasia	43/211 (20.5%)	51.2	0.78
<i>Submucosal layer</i>		104/210 (49.3%)	52.9	0.30
	Neutrophilic Infiltrate	16/210 (7.6%)	62.5	0.27
	Lymphoplasmocytic Infiltrate	84/210 (40.0%)	53.6	0.31
	Plexitis	81/210 (38.6%)	48.1	0.79
	Nervous Hyperplasia	17/210 (8.1%)	52.9	0.75
	Fibrosis	71/210 (33.8%)	59.1	0.04
<i>Subserosal and muscularis layer</i>		92/211 (43.6%)	50.5	0.78
	Lymphoplasmocytic Infiltrate	58/206 (28.2%)	60.3	0.04
	Fibrosis	22/206 (10.7%)	59.1	0.33
	Myenteric Plexitis Grade 2	74/211 (35.1%)	50.0	0.77
	Myenteric Plexitis Grade 3	38/211 (18.0%)	50.0	0.92
MACROSCOPIC DATA				

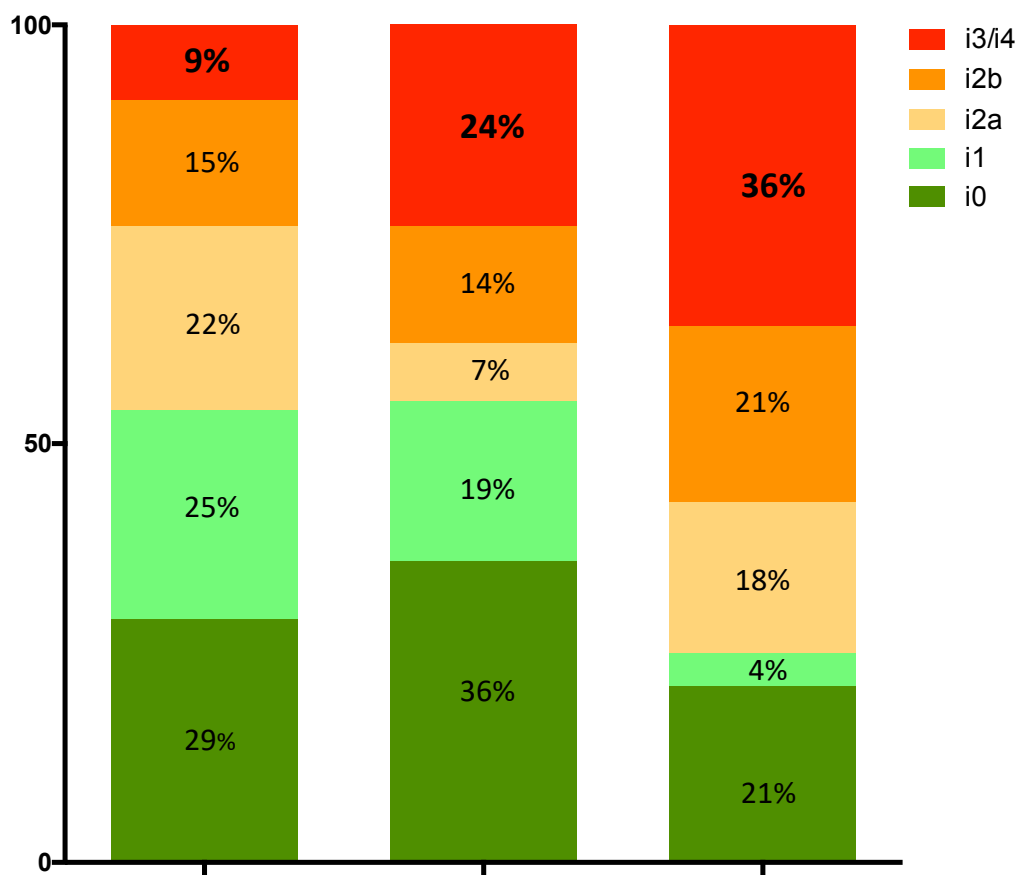
<i>Margin macroscopically affected</i>	44/211 (20.9%)	56.1	0.31
	Median in non recurrent patients (cm)	Median in recurrent patients (cm)	<i>p</i>
Length of ileal resection	19.0	18.8	0.96
Distance between first macroscopic ulceration and ileal margin	4.0	4.0	0.43

	Correlation with endoscopic recurrence (multivariate analysis)			Correlation with clinical long term recurrence (multivariate analysis)		
	<i>Odds Ratio</i>	<i>95% CI</i>	<i>p</i>	<i>Odds Ratio</i>	<i>95% CI</i>	<i>p</i>
Age	0.98	0.95 – 1.01	0.082	1.01	0.99 – 1.03	0.320
Gender (male)	2.31	1.26 – 4.32	0.008	1.39	0.85 – 2.28	0.189
Previous surgery	5.46	2.22 – 14.57	< 0.001	1.86	1.01 – 3.27	0.046
Active smokers	2.78	1.47 – 5.39	< 0.001	1.69	1.15 – 2.95	0.011
Postoperative anti-TNF	0.42	0.21 – 0.84	0.016	NA	NA	NA
Extradigestive manifestations	NA	NA	NA	2.11	1.19 – 3.63	0.010
CD transmural lesions	3.83	1.47 – 11.05	0.008	2.04	1.09 – 3.99	0.026



A

Endoscopic Recurrence Rate



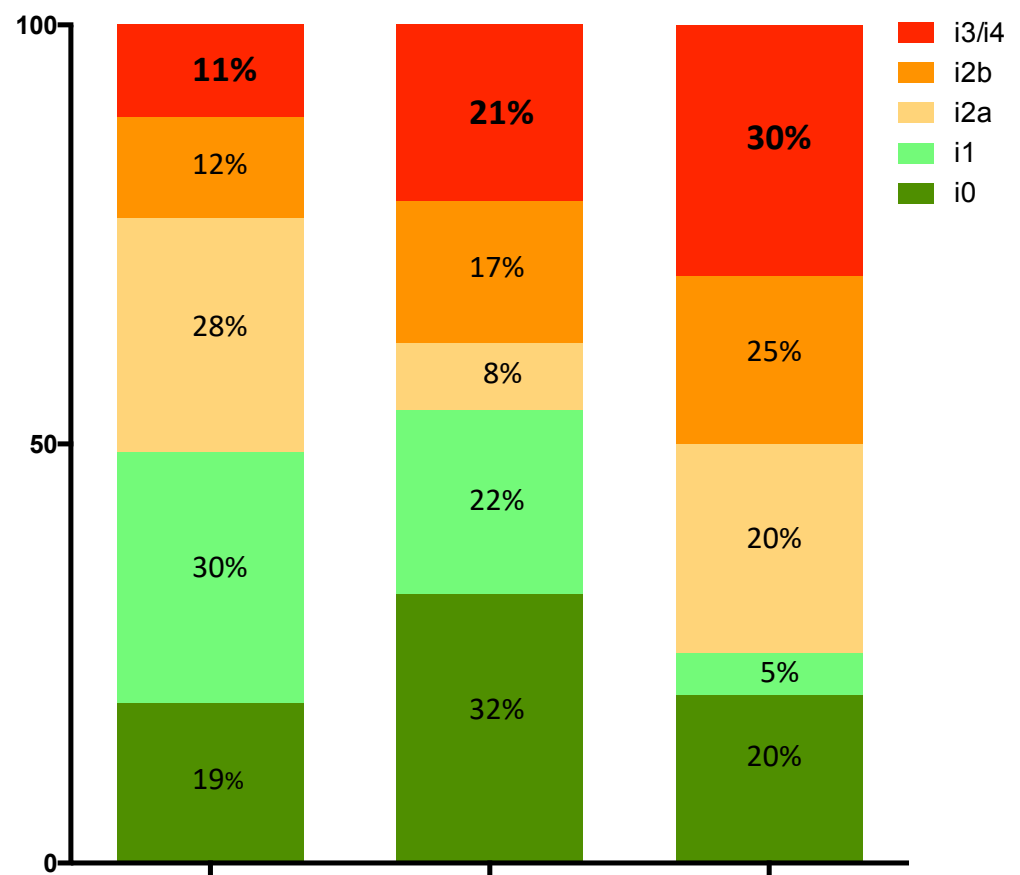
No lesion (n=65)

CD lesions but not transmural (n=113)

CD transmural lesions (n=28)

Histopathological lesions at ileal resection
margin**B**

Endoscopic Recurrence Rate

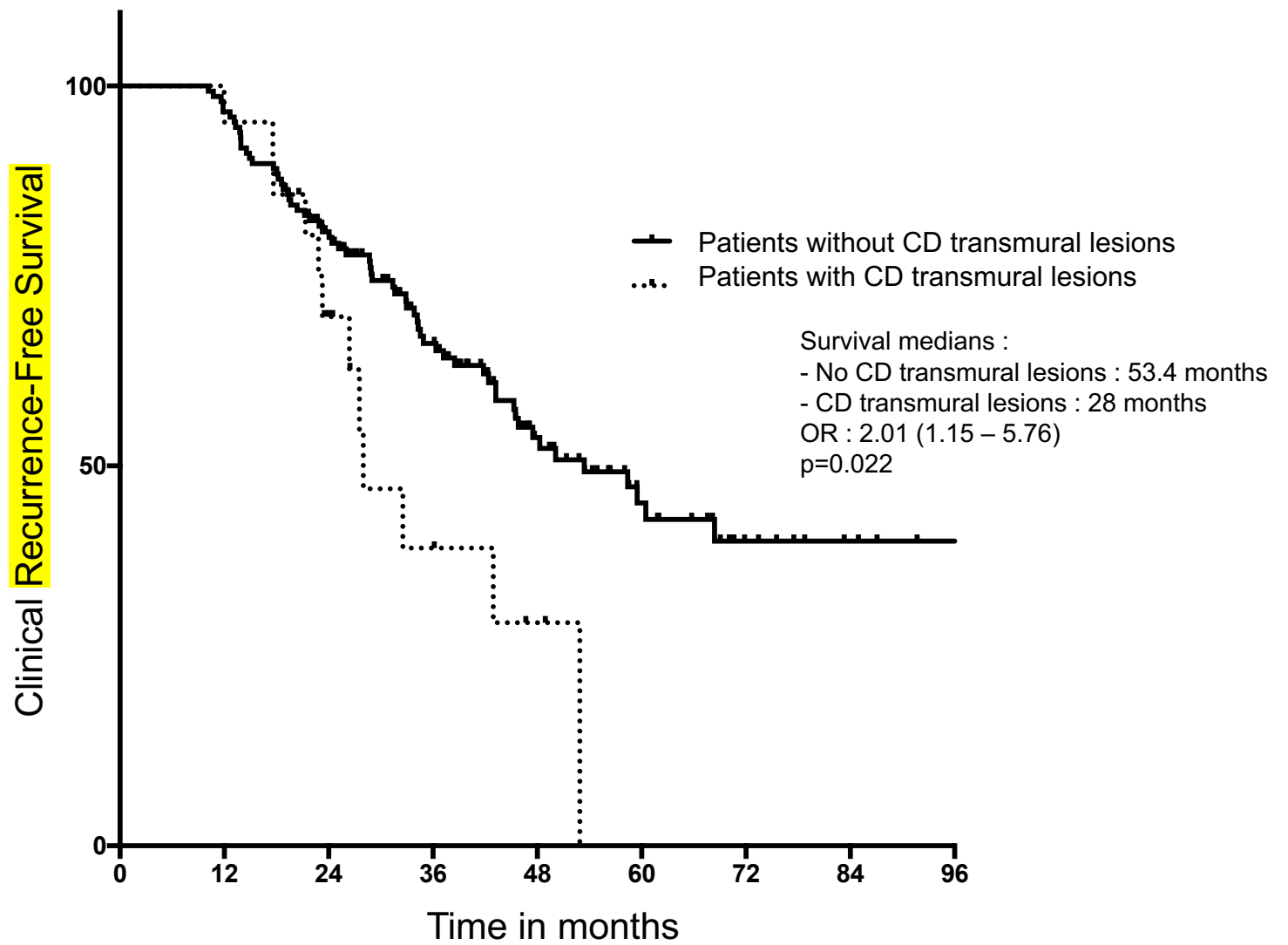


No lesion (n=43)

CD lesions but not transmural (n=88)

CD transmural lesions (n=20)

Histopathological lesions at ileal resection
margin
Patients without post-operative biotherapy



n: Without CD transmural lesions	147	143	110	72	39	22	10	5	2
n: With CD transmural lesions	21	21	13	6	3				

Need to Know

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

Findings: In patients with CD, transmural lesions at the ileal margin were associated with increased risk of post-operative disease recurrence.

Implications for Patient Care: Histologic features of the ileal margin should be included when physicians make decisions about post-operative therapy.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Flow chart

Supplementary Figure 2: Ven diagram: 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. 30.8% had a normal ileal margin.

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 one layer of the intestinal wall. Two layers: lesions affecting at least two layers of the intestinal wall. CD transmural lesions: presence of mucosal ulceration or cryptitis, submucosal fibrosis and lymphoplasmocytic infiltrate in the subserosa. Red points stand for patients who experienced endoscopic recurrence and blue points for patients who did not. Correlation test performed using Spearman's rank correlation test.