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

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

3  
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64

65 **ABSTRACT**  
66

67 **BACKGROUND AND AIMS:** Different types of histologic lesions at the ileal margin,  
68 detected by histology, have been associated with increased rates of recurrence after  
69 ileocaecal surgery in patients with Crohn's disease (CD). We aimed to characterize  
70 histologic features of the ileal margin and to evaluate their association with disease  
71 recurrence. **METHODS:** We collected histologic data from 211 patients with ileal or  
72 ileocolonic CD who underwent ileocolonic resections at hospitals in France from  
73 September 2010 through December 2016. Ileal margins were analyzed. Early  
74 endoscopic recurrence was defined by a Rutgeerts score of  $\geq 2$  or more, 6 months  
75 after surgery. We also collected data from 10 adults with healthy ileum who  
76 underwent ileocecal resection for colonic tumors (controls). Clinical relapse was  
77 defined by CD-related symptoms confirmed by imaging, endoscopy, therapy  
78 intensification, CD-related complication, or subsequent surgery. **RESULTS:** Six  
79 months after surgery, 49% of patients had endoscopic recurrence; 5 years after  
80 surgery, 57% of patients had clinical relapse. Ileal margins were macroscopically  
81 affected in 20.9% of patients. CD transmural lesions at the margin (defined by  
82 mucosal ulceration or cryptitis, submucosal fibrosis and lymphoplasmocytic infiltrate  
83 of the subserosa) were observed in 13.6% of patients. Endoscopic recurrence was  
84 observed in 75% of patients with CD transmural lesions vs 46% of patients without  
85 ( $P=.005$ ). In multivariate analysis, CD transmural lesions at the margin were  
86 independently associated with early endoscopic recurrence (OR, 3.83; 95% CI, 1.47-  
87 11.05;  $P=.008$ ) and clinical recurrence (OR 2.04; 95% CI, 1.09-3.99;  $P=.026$ ).  
88 **CONCLUSION:** In patients with CD, transmural lesions at the ileal margin were  
89 associated with an increased risk of post-operative recurrence. Histologic features of  
90 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

## 114 INTRODUCTION

115

116 Despite recent advances in the medical management of Crohn's Disease  
117 (CD), more than two thirds of patients still require intestinal resection during the  
118 course of their disease (1-3). Furthermore, surgery is not curative and post-operative  
119 recurrence occurs in the majority of patients. Early post-operative endoscopic  
120 recurrence, which is mainly observed at the anastomosis or in the neo-terminal  
121 ileum, precedes clinical recurrence and its severity predicts the long-term clinical  
122 outcome (4). Several risk factors have been reported to increase post-operative  
123 recurrence, including active smoking, penetrating disease, history of perianal  
124 disease, previous intestinal surgery and male gender (5-7). There is still a need for  
125 factors that can accurately risk-stratify patients (8).

126 Macroscopically involved ileal margin could increase post-operative  
127 recurrence risk but this assumption was pushed aside in 1996 by Fazio et al with the  
128 results of a randomized controlled trial showing no benefit of extended resection  
129 (clearance of 12 cm versus 2 cm of normal ileal margin) (9). Retrospective studies  
130 showed conflicting results regarding the impact of CD microscopic lesions on the ileal  
131 margin (mainly granuloma or active inflammation) on post-operative recurrence (10-  
132 13). Several publications have described an association between plexitis and  
133 endoscopic, clinical or surgical recurrence. Plexitis is classically defined by the  
134 presence of inflammatory cells in the myenteric or submucosal plexi (14). There were  
135 diverse definitions of plexitis and various endpoints were used (15-21). More  
136 recently, a cohort analysis of consecutive CD patients undergoing a first ileocaecal  
137 resection in two specialized centers found that microscopic involvement of the ileal  
138 margin, with the presence of granuloma, ulcers or transmural inflammation, was an  
139 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).



146 **MATERIALS AND METHODS**

147

148 ***Post-operative cohort and patient selection***

149 The present work was undertaken on a prospective multicenter study  
150 performed by the REMIND group that aimed at identifying predictors of early post-  
151 operative endoscopic recurrence (7). Patients were included between September  
152 2010 and December 2016. Inclusion criteria were: age >18 years, ileal or ileocolonic  
153 CD and indication of CD-related intestinal surgery (ileocolonic resection). A post-  
154 operative treatment was recommended according to a pre-established algorithm,  
155 based on the following risk factors: current smoking, previous bowel resection,  
156 penetrating phenotype and active perianal disease (detailed in 7). All patients had, 6  
157 months after surgery, a colonoscopy to assess the endoscopic recurrence according  
158 to the Rutgeerts score (23). From this cohort, we selected patients who had a  
159 paraffin-embedded ileal margin sample available for histological analysis. Margins  
160 with poor quality paraffin embedding were excluded. All patients provided an  
161 informed written consent. The study was approved by AFSAPS (IDRCB: 2009-  
162 A00205-52), the French Ethic Committee - Hôpital Saint-Louis (CPP 2009/17) and  
163 declared to clinicaltrials.gov (NCT03458195).

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

168

169

170

171 ***Data collection***

172 Clinical parameters were collected prospectively at time of surgery and at  
173 post-operative endoscopy. They included demographical data (gender, age at  
174 diagnosis, age at surgery, disease duration at surgery, smoking status at surgery),  
175 clinical data, complication and surgical indication, anoperineal lesions, previous  
176 history of intestinal resection, extra-digestive manifestations, treatment history  
177 including previous exposure to immunosuppressants (azathioprine, 6-MP or  
178 methotrexate) and anti-TNF agents.

179 An endoscopy was performed 6 months after surgery. Endoscopic data were  
180 collected prospectively. All lesions were reported in all segments, including the  
181 anastomotic region and the neo-terminal ileum. The physician who performed the  
182 colonoscopy evaluated the Rutgeerts score for each patient. Two physicians (MA  
183 and CA) checked the colonoscopy's report blinded to histological analysis and post-  
184 operative treatment.

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

194

195

196 ***Histopathological analysis***

197 Paraffin blocks of the ileal margin were cut into a full-thickness 3- $\mu$ m section  
198 and stained with hematein eosin saffron (HES). Each section was jointly analyzed by  
199 two expert pathologists (DCH and CG) blinded to the baseline detailed clinical data  
200 and to the post-operative outcomes of the patients. An analysis grid was built to  
201 assess CD-related lesions affecting each intestinal wall layer (mucosa, submucosa,  
202 and subserosa/muscularis).

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

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

212

213 ***Evaluated outcomes***

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

216 Clinical recurrence was defined by: (i) CD-related clinical manifestations  
217 confirmed either by endoscopy (Rutgeerts score  $\geq$  i2 or severe colonic lesions in one  
218 of the follow-up colonoscopies), imaging (active lesions of the small bowel or colon  
219 on an examination performed by the center referring radiologist) or therapeutic  
220 intensification (treatment optimization or drug switch), (ii) CD-related complications

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

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

225

## 226 ***Statistical analyses***

227 Descriptive statistics were calculated for all quantitative variables as medians  
228 with their respective interquartile ranges (IQR). Correlation between histology and  
229 recurrence, for each item of the analysis grid, was evaluated by both univariate and  
230 multivariate analyses. Chi-2 and Logistic regression were respectively used for binary  
231 variables. For continuous data (lengths in centimeters), Mann-Whitney test was used.  
232 Sensitivity, specificity, positive and negative predictive values were calculated using  
233 standard formulas. Spearman's rank correlation test was used when necessary to  
234 evaluate the correlation between variables.

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

240 All statistical analyses were two-tailed and a p value of less than 0.05 was  
241 considered as statistically significant. To perform these analyses, R software ® (3.2.2  
242 version) and PrismGraphPad ® were used.

243 **RESULTS**

244 ***Patients' characteristics at surgery and during follow-up***

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

249 Median age at surgery was 36.3 years (IQR: 30.7-47.5) and median disease  
250 duration was 6.3 years (IQR: 1.8-11.9). Ninety-two (44%) patients were male, 67  
251 (32%) active smokers and 38 (18%) had a previous ileocaecal resection in their past  
252 medical history (**Table 1**). Post-operative treatment was given to 102 (48%) patients,  
253 including an anti-TNF treatment in 52 (25%) patients.

254 Post-operative endoscopy was performed in all patients with a median time  
255 between resection and colonoscopy of 6.8 months. One hundred and four patients  
256 (49%) had an endoscopic recurrence (i.e. Rutgeerts score  $\geq$  i2) and 43 (20%)  
257 patients had severe lesions (i.e. i3 or i4). One hundred and seven (51%) patients had  
258 no endoscopic recurrence including 68 (32%) without any lesion (i0).

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

263 Median follow-up was 3.8 years (IQR: 2.55-5.41). Seventy-five (45%) patients  
264 experienced clinical relapse during the follow-up. Cumulative clinical recurrence rate  
265 was 5%, 37% and 57% at 1, 3 and 5 years respectively. At the end of the follow-up, 4  
266 (2%) patients underwent a CD-related subsequent surgery (3 ileocolonic resections,  
267 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

293 none of the three histological features defining transmural impairment (Spearman's  
294 rank correlation test:  $r=-0.476$ ;  $p<0.001$ ) (**Supplementary Figure 3**).

295

### 296 ***Correlation with endoscopic recurrence***

297 The ileal resection length, the macroscopic involvement of the ileal margin and  
298 the distance between the ileal margin and the first macroscopic CD ulceration were  
299 not correlated with endoscopic recurrence ( $p=0.96$ ,  $p=0.31$  and  $p=0.43$  respectively).

300 In univariate analysis, two histological lesions were significantly associated  
301 with endoscopic recurrence: submucosal fibrosis (recurrence rate: 59.1% vs. 44.6%,  
302 OR=1.80, 95%CI [1.01-3.21],  $p=0.04$ ) and lymphoplasmocytic infiltrate of the  
303 subserosa (recurrence rate: 60.3% vs. 44,6%, OR=1.89, 95%CI [1.01-3.51],  $p=0.04$ )  
304 (**Table 2**). A trend towards recurrence was also found in patients displaying mucosal  
305 ulceration or cryptitis indicative of active CD (recurrence rate: 58.3% vs. 45.7%,  
306 OR=1.66, 95%CI [0.91-3.07],  $p=0.09$ ) (**Table 2**). Submucosal or myenteric plexitis  
307 were not associated with endoscopic recurrence.

308 Based on this univariate analysis, we defined *a posteriori* the item “CD  
309 transmural lesions” by the presence of mucosal ulceration or cryptitis, submucosal  
310 fibrosis and lymphoplasmocytic infiltrate of the subserosa. This histological feature  
311 was observed in 28 patients (13.6%) out of the 206 samples in which these three  
312 lesions could be assessed. These 28 patients had a significant increased risk of  
313 endoscopic recurrence when compared to those without this histological feature  
314 ( $p=0.005$ ) (**Figure 2a**). Severe recurrence (i.e. score i3 or i4) was also more frequent  
315 in patients with CD transmural lesions on margin ( $p=0.03$ ) (**Figure 2a**). The sensitivity  
316 and specificity of the presence of CD transmural lesions for the risk of endoscopic

317 recurrence was 20% and 93% respectively with an accuracy of 63%. Consequently,  
318 the positive and negative predictive values were of 75% and 53% respectively.

319 Only 8 of the 28 patients who had CD transmural lesions received post-  
320 operative anti-TNF. Only 2 were i0 and 6 had a recurrence ( $\geq$  i2). In patients with no  
321 post-operative biotherapy, CD transmural lesions were also associated with  
322 endoscopic recurrence ( $p=0.03$ , **figure 2b**).

323 We integrated the selected lesions indicative of CD transmural lesions in a  
324 multivariate analysis with the clinical parameters associated with an increased risk of  
325 endoscopic recurrence in our cohort (i.e. male gender, previous intestinal surgery,  
326 active smoker at surgery and post-operative preventive anti-TNF treatment (7)). CD  
327 transmural lesions remained independently associated with endoscopic recurrence  
328 (OR: 3.83, 95%CI [1.47-11.05],  $p=0.008$ ) (**Table 3**).

329

### 330 ***Correlation with long-term clinical outcome***

331 In univariate analysis, clinical parameters significantly associated with long-  
332 term clinical recurrence were: active smoking at time of surgery ( $p=0.007$ ), previous  
333 intestinal surgery ( $p=0.012$ ), and presence of extra-intestinal manifestations at  
334 surgery ( $p=0.049$ ). CD transmural lesions at the ileal margin were also associated  
335 with a significantly poorer outcome ( $p=0.022$ ) (**Figure 3**).

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

339



340 **DISCUSSION**

341 This prospective study is, to our knowledge, the largest analyzing the impact  
342 of macroscopic and microscopic CD involvement of the ileal resection margin on  
343 endoscopic and clinical outcomes after ileocaecal resection. CD transmural lesions at  
344 ileal margin were strongly correlated with both early post-operative endoscopic  
345 recurrence and long-term clinical outcome independent of other risk factors.  
346 Conversely, we didn't find any correlation between reported macroscopic ulceration  
347 noticed on the ileal margin and recurrence.

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

352 A recent literature review emphasized the crucial need of new studies  
353 assessing the involvement of both macroscopic and microscopic lesions in CD  
354 recurrence after surgery (24). Two recent publications found an association between  
355 microscopic lesions at ileal margins and recurrence in both adult and pediatric  
356 patients (22, 25). However, no detail on the type of lesions and their location in the  
357 intestinal wall were provided. Herein, two experts pathologists assessed each CD  
358 histological lesions, including plexitis, using a reproducible grid. Thus, in contrast to  
359 recent literature on the subject (15-21), we considered all CD-related lesions  
360 potentially found in the whole bowel wall through a complete and reproducible  
361 analysis rather than focusing on plexitis alone. In our cohort, plexitis, whatever its  
362 severity, was not associated with recurrence. Interestingly, most of the patients with  
363 grade 3 MP had significant lesions in other layers, while few patients (n=6) had  
364 plexitis only.

365 We hypothesized that some histological lesions could be more relevant than  
366 others and selected them from our statistical analysis. In univariate analysis, two  
367 lesions were significantly associated with endoscopic recurrence - fibrosis of the  
368 submucosa and lymphoplasmocytic infiltrate of the subserosa - reflecting sequelae  
369 of previous CD flares or persistent chronic inflammation. Moreover, mucosal lesions  
370 such as ulceration or cryptitis were also associated with a trend towards recurrence.  
371 These 3 lesions had a similar impact on post-operative recurrence (odds ratios for  
372 ulceration/cryptitis, fibrosis of the submucosa and lymphoplasmocytic infiltrate of the  
373 subserosa were 1.66 [0.91-3.07], 1.80 [1.01-3.21] and 1.89 [1.01-3.51] respectively).  
374 Thus, we used the combination of these 3 CD-related lesions, each affecting one  
375 layer of the bowel wall, to define intense and active CD transmural involvement of the  
376 ileal margin, which was strongly associated with recurrence. This finding highlights  
377 that, rather than isolated microscopic lesions, deep CD-related tissue injury of the  
378 ileal margin could influence recurrence risk. These patients could benefit from an  
379 immediate post-operative treatment.

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

388 CD is characterized by discontinuous patchy or extensive transmural  
389 inflammation with skip lesions. Recurrence remains frequent despite normal

390 resection margins. Indeed, 46% of patients with normal ileal margin experienced  
391 endoscopic recurrence, which was even severe in 10% (i3 or i4). Histological  
392 evaluation of the margin has therefore a low sensitivity for prediction of post-  
393 operative recurrence.

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

404

405 In conclusion, we show in this prospective multicenter cohort study that CD  
406 transmural histological lesions at the ileal margin of ileocaecal resection specimens  
407 in CD were associated with an increased risk of endoscopic and clinical recurrence.  
408 Pathologists should systematically detail in their reports the analysis of the ileal  
409 margin of resection notifying the presence of CD transmural lesions  
410 (ulceration/erosion, fibrosis and lymphoplasmocytic infiltrate). A systematic post-  
411 operative treatment could be proposed to patients with transmural histological  
412 lesions, even in absence of other risk factors. The better post-operative treatment to  
413 initiate to these patients remains to be determined; ClinicalTrials.gov number,  
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520 **TABLE LEGENDS**

521

522 **Table 1: Patients' characteristics at time of surgery**

523

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

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

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

534 Pyloric Metaplasia=metaplastic glands of the ileal mucosa

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

536 Neutrophilic infiltrate=nest of at least 50 neutrophils in the submucosae at x400

537 magnification; Lymphoplasmocytic infiltrate=nest of at least 50 lymphocytes or

538 plasma cells at x400 magnification; Plexitis=inflammatory cells around or inside

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

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

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

542 least 10 immune cells);

543 Nervous hyperplasia=enlarged nervous bundles visible at x100 magnification;

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

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

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

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577 **FIGURES LEGENDS**

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580 **Figure 1: Pathological analysis of the whole ileal margin on surgical specimen**  
581 **and on section stained by hematein-eosin-saffron.** (A)-Macroscopic view of an  
582 opened ileocaecal resection specimen with mucosal ulcerations visible on ileal  
583 proximal margin (arrow) (B)-Microscopic view of the whole ileal margin section  
584 showing a bowel wall with CD-related lesions affecting the 3 layers (mucosal,  
585 submucosal and subserosal layers in circles). (C)-Fibrosis observed in the  
586 submucosa. (D)-Lymphoplasmocytic infiltrate observed in the subserosa.

587

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

591 No lesion: patients with normal ileal margin. CD lesions but not transmural: patients  
592 with ileal margin affected but without CD transmural lesions defined by the presence  
593 of mucosal ulceration or cryptitis, submucosal fibrosis and lymphoplasmocytic  
594 infiltrate of the subserosa. CD transmural lesions: patients with these three lesions on  
595 ileal margin. A: Whole cohort. B: Patients with no post-operative biotherapy.

596

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

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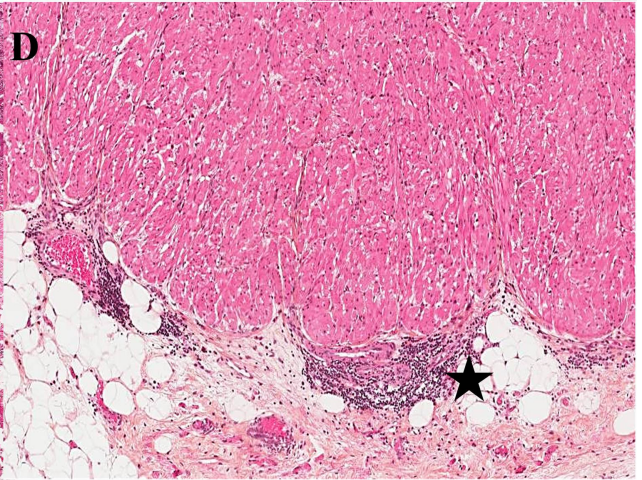
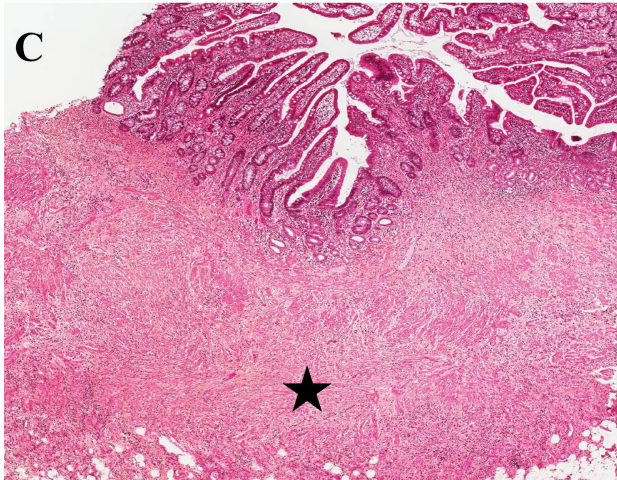
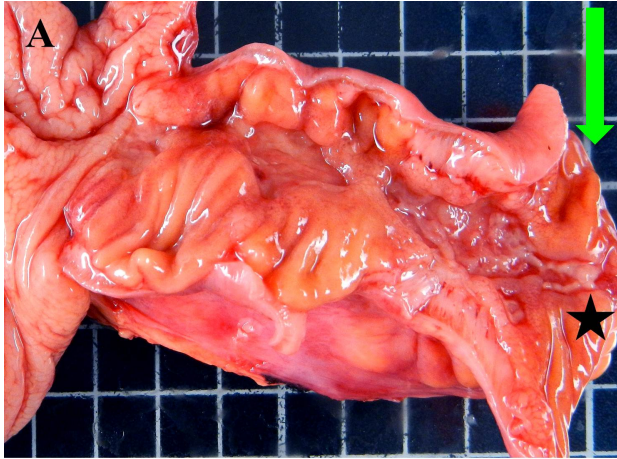
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| <b>Patients' characteristics at time of surgery</b>        | <b>Patients n=211 (%)</b> |
|--|---------------------------|
| Men  | 92 (44%)                  |
| Median age (y, IQR)  | 36.3 (30.7 – 47.5)        |
| Age at Crohn's Disease diagnosis (Montreal classification) | 19 (9%)                   |
| - ≤16 years (A1)   | 170 (81%)                 |
| - 17 - 40 years (A2)                                       | 22 (10%)                  |
| - >40 years (A3)   |                           |
| 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  | 67 (32%)                  |
| - Active smoker  | 13 (6%)                   |
| - Smoking cessation at surgery                             | 131 (62%)                 |
| - Non smoker   |                           |
| Previous intestinal resection                              | 38 (18%)                  |
| - <10 years  | 14 (7%)                   |
| Number of previous resection(s)                            | 173 (82%)                 |
| - 0  | 27 (13%)                  |
| - 1  | 7 (3%)                    |
| - 2  | 4 (2%)                    |
| - 3  |                           |
| Surgical indication  | 115 (55%)                 |
| - Stricturing complication                                 | 83 (39%)                  |
| - Penetrating complication                                 | 13 (6%)                   |
| - Failure of drug therapy                                  |                           |
| Disease location (Montreal classification)                 | 132 (63%)                 |
| - Ileal (L1)   | 79 (37%)                  |
| - Ileocolonic (L3)   | 50 (24%)                  |
| - Anoperineal lesion                                       |                           |
| Extra-digestive symptoms                                   | 40 (19%)                  |
| - Joint manifestations                                     | 12 (6%)                   |
| - Skin manifestations                                      | 4 (2%)                    |
| - Eye manifestations                                       |                           |
| Previous exposure to anti-TNF therapy                      | 134 (64%)                 |
| Anti-TNF therapy within 3 months before surgery            | 107 (51%)                 |
| Previous exposure to thiopurines                           | 145 (69%)                 |
| Thiopurines within 3 months before surgery                 | 60 (28%)                  |

| <b>MICROSCOPIC DATA</b>                       | <b>Histological lesions</b>  | <b>Prevalence (affected/analyzed)</b> | <b>Endoscopic recurrence (%)</b> | <b><i>p</i></b> |
|---|------------------------------|---------------------------------------|----------------------------------|-----------------|
| <b>Normal margin</b>                          |                              | <b>65/211 (30.8%)</b>                 | 46.2                             | 0.54            |
| <b>Margin affected</b>                        |                              | <b>146/211 (69.2%)</b>                | 50.7                             | 0.54            |
| <b><i>Mucosal layer</i></b>                   |                              | <b>111/211 (52.6%)</b>                | 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%)                        | <b>58.3</b>                      | <b>0.09</b>     |
|   | Villous Atrophy              | 20/211 (9.5%)                         | 55.0                             | 0.59            |
|   | Pyloric Metaplasia           | 43/211 (20.5%)                        | 51.2                             | 0.78            |
| <b><i>Submucosal layer</i></b>                |                              | <b>104/210 (49.3%)</b>                | 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%)                        | <b>59.1</b>                      | <b>0.04</b>     |
| <b><i>Subserosal and muscularis layer</i></b> |                              | <b>92/211 (43.6%)</b>                 | 50.5                             | 0.78            |
|   | Lymphoplasmocytic Infiltrate | 58/206 (28.2%)                        | <b>60.3</b>                      | <b>0.04</b>     |
|   | 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            |
| <b>MACROSCOPIC DATA</b>                       |                              |                                       |                                  |                 |

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

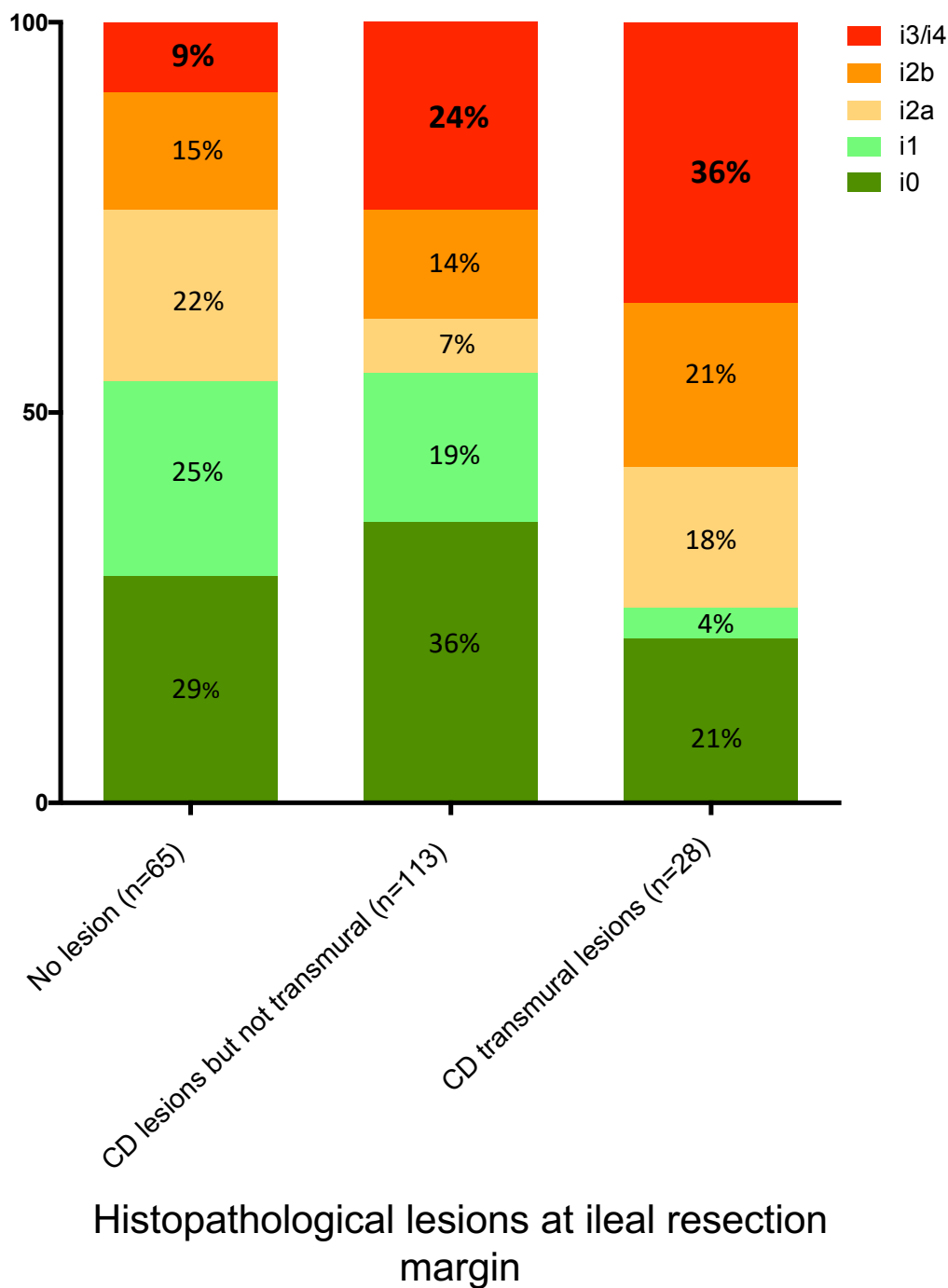
|                               | Correlation with endoscopic recurrence (multivariate analysis) |               |          | Correlation with clinical long term recurrence (multivariate analysis) |               |          |
|-------------------------------|--|---------------|----------|--|---------------|----------|
|                               | <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    |



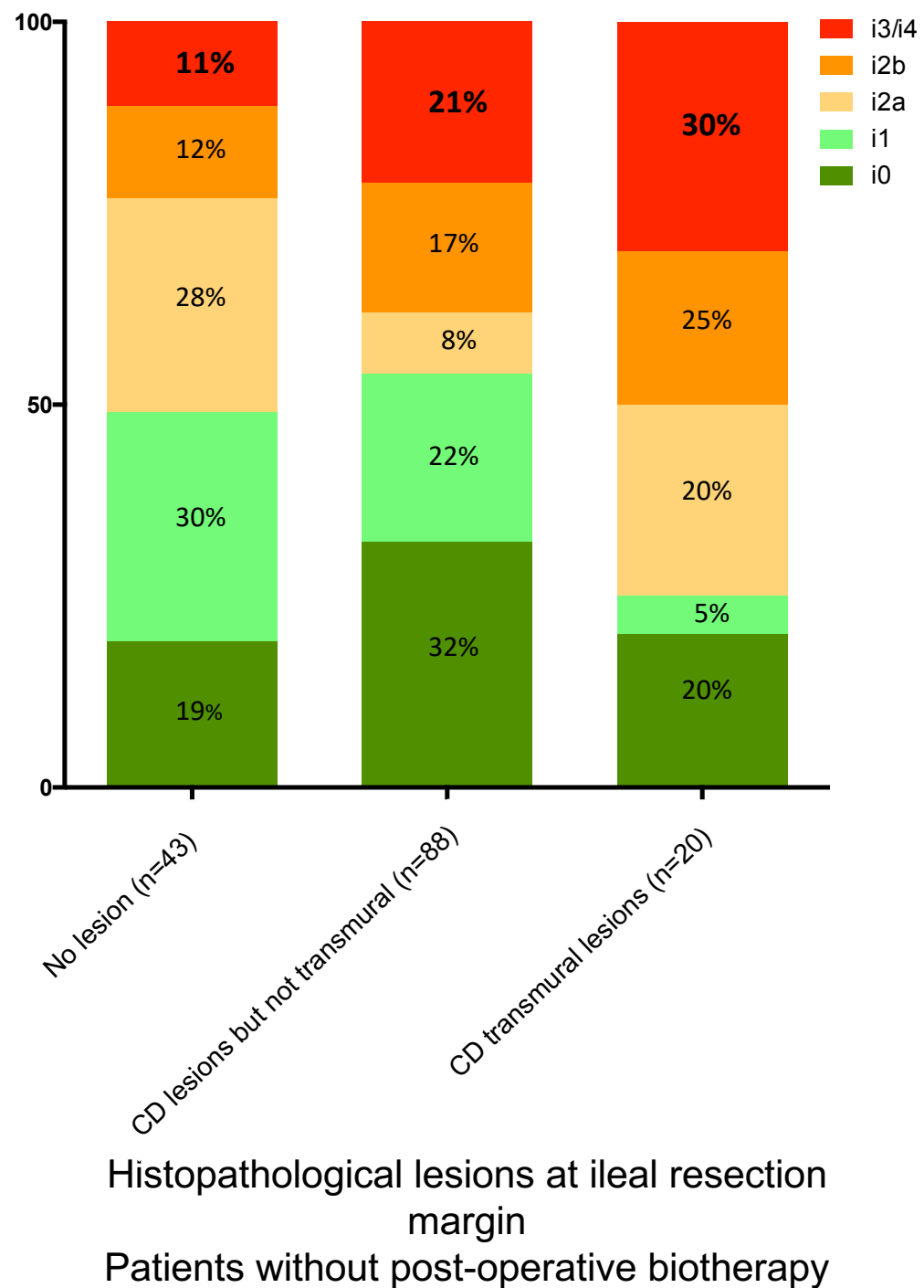


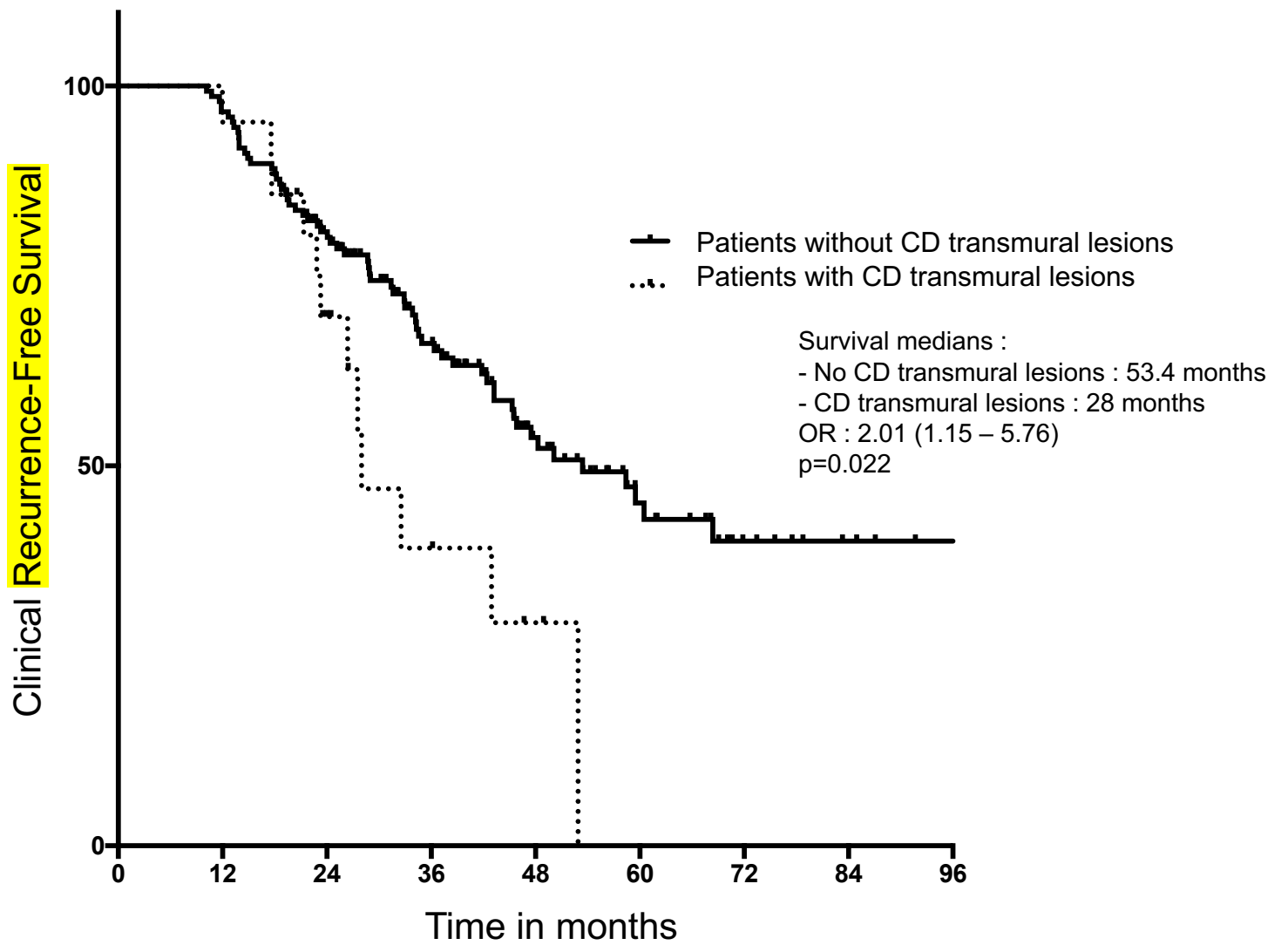
**A**

Endoscopic Recurrence Rate

**B**

Endoscopic Recurrence Rate





|                                  |     |     |     |    |    |    |    |   |   |
|----------------------------------|-----|-----|-----|----|----|----|----|---|---|
| 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.