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

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Aim: Ileocolonoscopy allows early detection of recurrence after surgical resection for Crohn’s disease (CD). Plexitis, defined as presence of inflammatory cells in or around enteric ganglia or nerve bundles, in the proximal surgical margin has been associated with an increased overall recurrence risk. We investigated prospectively whether plexitis can predict endoscopic recurrence (ER) in a consecutive cohort of CD patients undergoing ileocolonic resection.

Methods: All CD patients undergoing ileocolonic resection in our institution between October 2009 and December 2012 were eligible for this study. Clinical data were obtained prospectively from the patients’ files, and biopsies from the proximal surgical margins were analysed immunohistochemically for inflammation at the myenteric and submucosal plexus [lymphocytes, mast cells, eosinophils]. The degree of plexitis was correlated with the presence of ER at 6 months, defined as a modified Rutgeerts’ score of ≥ i2b. Multivariate models were developed and tested to predict posterior probability of ER.

Results: A total of 74 patients were included. Six months after ileocolonic resection, 50% showed ER. Known risk factors such as penetrating disease, previous resections, and active smoking, showed no relation with ER. On the other hand, submucosal lymphocytic plexitis was associated with ER (p = 0.020). The predictive value of lymphocytic cell count increased with more extensive biopsy sampling and with application of immunohistochemistry.
Conclusions: Submucosal lymphocytic plexitis in the proximal surgical margin was significantly related with a higher risk for ER after ileocolonic resection. These data support development of a postoperative prevention trial with vedolizumab, which may block lymphocytic trafficking in the postoperative bowel.

Key Words: Endoscopy; pathology; surgery

1. Introduction
Despite improvements in medical therapy for Crohn’s disease [CD], almost two-thirds of the patients require surgery and almost half of these patients will be operated within 5 years after diagnosis.1-3 Unfortunately, the majority will experience postoperative endoscopic recurrence [ER] with ulcerative lesions typically occurring in the neo-terminal ileum proximal to the ileocolonic anastomosis.4,5 The severity of these lesions witnessed on ileocolonoscopy performed as early as 6 months postoperatively, predicts the recurrence of clinical symptoms and need for consecutive surgery.

Since endoscopy is a particular burden for the postoperative patient, accurate prediction of postoperative ER would be useful to guide initiation of postoperative prophylactic therapy. Several risk factors have already been reported to be associated with postoperative recurrence, i.e. penetrating disease, previous bowel resection, and active smoking.4 Elevated C-reactive protein [CRP] level at time of surgery, and presence of NOD2/CARD15 mutations, have also been suggested as risk factors although not widely replicated.6

Early histological studies show conflicting data regarding the predictive role of granulomas and active inflammation.7,8 However, the presence of inflammatory cells in the myenteric and submucosal plexus at the proximal section margin [plexitis], even without macroscopically visible lesions, has increased investigators’ interest in this field.9 We were the first to study plexitis at the resection margins of ileocolonectomy specimens in a retrospective cohort analysis, and showed that the presence of myenteric plexitis of the proximal resection margin, defined as at least one inflammatory cell apposed to or within enteric ganglia or nerve bundles, was predictive for ER after ileocolonic surgery.10 The type of inflammatory cells [eosinophils, lymphocytes, plasma cells, or mast cells] was not informative in this study. Thereafter additional studies, applying different methodologies [haematoxylin-eosin [H&E], or immunohistochemistry [IHC]] and different endpoints [endoscopic, clinical, or surgical recurrence] reported variable results [Table 1].11-14 Since ER precedes clinical and surgical recurrence, prediction of ER would be most valuable in order to initiate prophylactic treatment.4

In this prospective study using immunohistochemistry, we aimed to investigate the predictive value of submucosal and myenteric plexitis at the proximal section margin for postoperative ER, in a large cohort of CD patients undergoing ileocaecal resection with ileocolonic anastomosis.

2. Materials and Methods

2.1. Patient selection
All CD patients eligible for an ileocaecal resection at the University Hospitals Leuven between December 2009 and October 2012 were included after informed consent. An ileocolonoscopy was performed 6 months after ileocaecal resection or closure of a temporary stoma. Patients in whom prophylactic therapy was initiated before postoperative ileocolonoscopy, or who received a permanent ileostomy, were excluded. All relevant clinical data were retrieved from patients’ medical files. Two separate control groups were included: 19 patients who underwent a total colectomy for ulcerative colitis [UC] and 19 patients undergoing a right hemicolectomy for a caecal adenocarcinoma. The study protocol was approved by the local ethical committee of the University Hospitals Leuven [ref B322201213950 – S53684].

Table 1. Previous studies on plexitis and Crohn’s disease recurrence.

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<tbody>
<tr>
<td><strong>Number of patients</strong></td>
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<tr>
<td>59 CD</td>
<td>171 CD</td>
<td>99 CD</td>
<td>67 CD</td>
<td>86 CD</td>
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<tr>
<td><strong>Type of inflammatory cells [staining]</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Eosinophils [H&amp;E]</td>
<td>Lymphocytes [H&amp;E]</td>
<td>Plasma cells [H&amp;E]</td>
<td>Plasma cells [H&amp;E]</td>
<td>Mast cells [H&amp;E]</td>
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<tr>
<td><strong>Evaluated plexus in proximal resection margin</strong></td>
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<tr>
<td>Myenteric + submucosal</td>
<td>Myenteric + submucosal</td>
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<td>Myenteric + submucosal</td>
<td>Myenteric</td>
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<tr>
<td><strong>Endpoint</strong></td>
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<tr>
<td>Endoscopic recurrence</td>
<td>Clinical recurrence</td>
<td>Clinical + surgical recurrence</td>
<td>Surgical recurrence</td>
<td>Clinical + surgical recurrence</td>
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<tr>
<td><strong>Predictive marker</strong></td>
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<td>≥ 1 inflammatory cell in the myenteric plexus</td>
<td>≥ 3 mast cells in the submucosal plexus</td>
<td>No correlation</td>
<td>≥ 1 eosinophil or &gt; 6 lymphocytes in the submucosal plexus</td>
<td>≥ 10 inflammatory cells/HPF in the myenteric plexus</td>
</tr>
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</table>

1Analyses based on the results for the most severely inflamed ganglion.
CD, Crohn’s disease; CD3, immunohistochemistry with anti-CD3 staining for lymphocytes; CD117, immunohistochemistry with anti-CD117 for mast cells; H&E, haematoxylin-eosin; HPF, high power field.
2.2. Histological examination

Soon after resection, surgical specimens were fixed in 4% formalin and transmural biopsies were embedded in paraffin following standard procedures. As part of this routine protocol, a 2 x 2 cm transmural biopsy was taken from the proximal section margin of each specimen. Three serial 5-µm thick slices were obtained from this block. One of the slides was stained with H&E and the remaining two slides were reserved for automated IHC (Leica Bond-Max, Leica Biosystems, Germany). A CD45 stain was performed for lymphocytes (Dako, Germany; RTU, antigen retrieval at pH 6, blocking of endogenous peroxidase, 10-min incubation time) and a tryptase stain for mast cells (Dako, Germany; 1/400 dilution, antigen retrieval at pH 6, blocking of endogenous peroxidase, 20-min incubation time).

Histological slides were analysed by three experienced pathologists [AT, KG, GDH] blinded for the clinical data. First, each proximal resection margin biopsy was investigated for the presence of typical CD lesions [granulomas, ulcerations, transmural inflammation, fistulas]. Next, inflammatory cells, both within and apposed to every ganglion of the myenteric and the submucosal plexus, were counted systematically. Eosinophils were counted on the H&E-stained slides, lymphocytes on CD45-stained slides and mast cells on tryptase-stained slides [Figure 1]. Only cells with a visible nucleus were included in the count results. A ganglion was defined as an accumulation of neuronal cell bodies and glial cells, with at least one neuronal cell body.

Additionally, 24 cases were evaluated for anti-integrin beta7 (ITGβ7) by manual immunohistochemistry on formalin-fixed, paraffin-embedded tissue of the proximal resection margin [Atlas Antibodies, Sweden; 1/1 000 dilution, antigen retrieval at pH 9, blocking of endogenous peroxidase, 30-min incubation time]. These 24 cases were selected based on a high count of lymphocytes in their proximal margin. The anti-ITGβ7 antibody stains lymphocytes, polymorphs, macrophages, and giants cell.

2.3. Assessment of postoperative recurrence

All patients underwent an ileocolonoscopy 6 months after ileocolonic resection. In patients requiring a temporary ileostomy, postoperative endoscopy was performed 6 months after closure of the ileostomy. Endoscopy was performed by experienced gastroenterologists [GVA, SV, MF], and postoperative ER was based on the Rutgeerts’ score. However, the original i2 grade from the Rutgeerts’ score was subdivided depending on the location of the lesions and associated risk for clinical recurrence, with i2a for lesions confined to the anastomosis [low risk] and i2b for occurrence of more than five aphthous lesions in the neo-terminal ileum [higher risk, Table 2]. Postoperative ER was defined as a Rutgeerts’ score.

Figure 1. a: A,B: CD45 stain in the submucosal and the myenteric plexus, respectively [arrows: lymphocytes apposed to ganglia, 400X]; C,D: haematoxylin-eosin [H&E] staining in the submucosal and the myenteric plexus [arrows: eosinophils apposed to and within a ganglion, respectively, 400X]; E,F: Tryptase staining in the submucosal and the myenteric plexus [arrows: mast cells apposed to ganglia, 630X].
of ≥ i2b as proposed by Gecse et al. Data on clinical and surgical recurrence were collected through chart review. Clinical recurrence was defined as the recurrence of CD-related symptoms, confirmed by objective signs of inflammation such as increase in C-reactive protein level, radiology, or endoscopy findings. Surgical recurrence was defined as the presence of clinical or endoscopic recurrence requiring another surgical resection.

2.4. Statistical analysis

Chi square tests were used for comparison of cell count data for all ganglia between CD patients and both control groups, and this for each location and each inflammatory cell type separately.

The association between clinical data and ER was investigated applying Fisher’s exact test, Mann-Whitney U test, and multiple logistic regression. The performance of previously described prediction models was assessed by reclassification of our patients into low- and high-risk groups, based on the count data for the most inflamed ganglion.

The predictive value of inflammatory cell count data [amount of inflammatory cells in the myenteric and submucosal plexus of the proximal resection margin] for ER was investigated using a negative binomial mixed model based on an application of Bayes’ rule. A Poisson-normal model was included as an alternative to verify the stability of the results.

Next, we evaluated the proportion of ganglia with [non-zero cell count] or without [zero cell count] presence of a specific cell type, and their predictive value on ER using binary analysis [binomial mixed model]. The discriminative ability of these models was quantified with area under the curve [AUC] analyses. Finally, Bayesian statistics were performed to evaluate the posterior probability of predicting ER based on inflammatory cell counts, taking into account the number of evaluated ganglia.

All analyses were performed using SAS software, version 9.2 [SAS Institute Inc., Cary, NC, USA]. A p-value < 0.05 was regarded as significant.

3. Results

3.1. Patient selection and characteristics

In total, 111 patients with CD gave written informed consent; 37 patients were excluded for various reasons [Supplementary Figure 1, available as Supplementary data at ECCO-JCC online]. Ultimately, 74 patients were included (30 male; median [IQR] age: 44.8 [33.4 – 52.9] years). In addition, 19 UC patients [15 male, median [IQR] age: 46.5 [38.2 – 54.4]] and 19 carcinoma controls [7 male, median [IQR] age: 69.0 [59.6 – 80.0]] were included. Baseline characteristics of the study group are listed in Table 3.

Table 2. Adapted postoperative endoscopic recurrence score.416

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description of endoscopic lesions</th>
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<tr>
<td>i0</td>
<td>Absence of lesions at the site of the anastomosis</td>
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<tr>
<td>i1</td>
<td>≤ 5 aphthous ulcers in the neo-terminal ileum [≤ 5 mm]</td>
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<tr>
<td>i2a</td>
<td>Lesions confined to the ileocolonic anastomosis [≤ 1 cm in length]</td>
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<tr>
<td>i2b</td>
<td>&gt; 5 aphthous ulcers in the neo-terminal ileum with normal mucosa between the lesions, or skip lesions</td>
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<tr>
<td>i3</td>
<td>Diffuse aphthous ileitis with diffusely inflamed intervening mucosa</td>
</tr>
<tr>
<td>i4</td>
<td>Diffuse ileal inflammation with larger ulcers [≥ 5 mm], nodules, and/or narrowing</td>
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3.2. Histology: general features

Microscopic inflammation at the proximal section margin was reported in 10 patients [14%] [transmural inflammation, n = 2; ulcerations, n = 6; epithelioid granulomas, n = 4].

Overall, mean numbers of myenteric and submucosal ganglia evaluated per patient and per stain were significantly different between CD patients and controls [Table 4]. However, the number of analysised ganglia was at least 10 in all cases.

In total, 23 778 inflammatory cells were counted within and apposed to ganglia. In CD, the maximal number of inflammatory cells per ganglion was higher for lymphocytes [namely, up to 14] than eosinophils [up to 7] and mast cells [up to 5]. Of note, the distribution of inflammatory cell counts per ganglion was very much skewed towards lower numbers [≥ 90% of the observed ganglia contained ≤ 2 inflammatory cells]. This was also illustrated by analysing the exact and relative number of inflammatory cells per ganglion [Table 5, and Table 6].

Overall, the mean count of all cell types was highest in the myenteric and submucosal plexus of patients with CD [Table 5].

3.3. Assessment of postoperative endoscopic recurrence [Rutgeerts’ score ≥ i2b]

Postoperative ER at 6 months was seen in 37 of the 74 patients [50%] [Figure 2]. Clinical recurrence rates were 30% at 1 year and 45% at 5 years. The median time to clinical CD recurrence was significantly shorter in patients with ER at Month 6 [5.8 [range: 2.6 – 37.0] vs 27.0 [range: 4.6 – 47.5] months, p = 0.001]. Surgical recurrence rates were 1% at 1 year and 7% at 5 years. All five patients [7%]
needing surgical intervention during follow-up, belonged to the group with postoperative ER.

3.4. Prediction of postoperative endoscopic recurrence

None of the predefined clinical variables [penetrating disease, smoking behaviour or previous bowel resection], were predictive for postoperative ER. Patients with typical CD lesions at the proximal section margin did not develop ER more often than patients without these lesions [60% vs 48%, p = 0.736].

When evaluating the overall cell count, the lymphocyte counts in the submucosalplexus showed a significantly different distribution between patients with and without ER [p = 0.020]. Also in the binary analysis classifying the count data for every ganglion as ‘zero’ [no inflammation] or ‘non-zero’ [inflamed ganglion], submucosal lymphocytes discriminated between CD patients with and without ER [p = 0.007].
3.5. Prediction of posterior probabilities of endoscopic recurrence as a function of cell counts

As shown in Figure 3A, subjects with identical proportions of inflamed ganglia displayed different probabilities of ER, depending on the number of evaluated ganglia. Indeed, the ratio of inflamed ganglia based on a higher number of ganglia had a higher predictive value than the same percentage based on a lower number of ganglia. This is also illustrated in Figure 3B where the posterior probabilities of future hypothetical patients are plotted as a function of the percentage of non-zero counts and the number of ganglia. The discriminatory power of a > 45%-inflamed ganglion cell count for ER increases proportionally to the number of evaluated ganglia.

Anti-integrin β7 immunohistochemistry

It seems that lymphocytes just migrating through the vessels are showing a lot of ITGβ7 positivity, whereas lymphocytes which are already present for a longer time [e.g. GALT] are losing this signal. When looking at the plexuses, both positive and negative lymphocytes are present in the proximal resection margin of CD patients [Figure 4].

4. Discussion

The frequently observed postoperative recurrence in patients with CD undergoing an ileocolic resection with ileocolonic anastomosis remains an important challenge in daily clinical practice. Although the postoperative prophylactic use of anti-tumour necrosis factor [TNF] agents seems beneficial, the related costs and possible adverse events warrant postoperative stratification and individualised therapy. It is necessary to identify those patients at higher risk for postoperative recurrence. Strikingly, only clinical features such as active smoking, penetrating disease, and previous resection are established risk factors of postoperative CD recurrence, and therefore are included in postoperative intervention trials. Other factors used to stratify postoperative therapy include active inflammation and young age at surgery. Unfortunately, these clinical and biological markers remain insufficient to guide postoperative therapy. In the present manuscript, none of the previously defined clinical risk factors for postoperative ER were confirmed, probably in part related to the cohort size. All other clinical or biochemical characteristics were not associated with postoperative ER.

In this manuscript, we explored the predictive value of the presence of inflammatory cells in the myenteric and submucosal plexus of the proximal section margin [also called plexitis]. Both the absolute and the relative number of lymphocytes in submucosal ganglia were predictive of postoperative ER. The posterior probability of postoperative ER was evident if more than 45% of the submucosal ganglia were affected by lymphocytes, and was more pronounced if more ganglia were available for histological evaluation.

For more than 50 years, histological analysis of resection specimens has been used in an attempt to predict postoperative CD recurrence. Several investigators focused on presence of granulomas and disease-free section margins; however, these studies reported conflicting results. In our data set, no relationship could be detected between the presence of typical CD lesions [including granulomata] and ER.

In this prospective study [the first of its kind], all submucosal and myenteric ganglia were histologically analysed in formalin-fixed paraffin-embedded material. Of note, patients who received postoperative prophylactic therapy [with thiopurines and/or biologicals] were excluded to obtain a more homogeneous population. Further, patients had to agree to undergo an ileocolonoscopy after 6 months. One may expect that this may have caused a bias. Patients without any risk and without any symptoms may have cancelled the planned postoperative endoscopic evaluation. A modified Rutgeerts’ score was used to evaluate ER after 6 months. The modified score was chosen since we have used this score in daily clinical practice already for several years. However no formal data are available yet, and ulcerative lesions confined to the anastomosis [score 12a] are considered to be less predictive of clinical recurrence compared with more than five aphthous lesions in the neo-terminal ileum [score 12b or higher]. In contrast to most other studies, we also included two subgroups of control patients, namely patients with UC and caecal adenocarcinoma.

Regarding the histological analysis, we evaluated only the proximal section margin of ileocolic resection specimens since plexitis is uncommon in the distal margin and postoperative ER tends to occur proximal of the anastomosis. To increase the yield for detecting inflammatory cells, we decided to use IHC rather than H&E alone, and analysed the presence of eosinophils, lymphocytes, and mast cells. Neutrophils were excluded because their presence may be indicative of surgical damage, i.e. unavoidable manipulation of the tissues during the operation. Plasma cells were likewise not taken into consideration as they are very rare around nerve plexuses in [grossly] uninfamed tissues.

This study showed the importance of evaluating not only the most affected ganglion, but also to assess the complete enteric nervous system at the proximal resection margin. The value to predict postoperative ER was indeed higher if more ganglia were evaluated. Only two of the previous studies looking into plexitis mentioned the number of analysed ganglia, and none of them required a minimal amount of ganglia to be present. In this study, we made two observations in this regard. First, we detected a difference in numbers of ganglia present in consecutive slides for single patients [data not shown]. The observation that more ganglia were counted when searching for plexitis, is probably explained by the better contrast on H&E stained slides. The alternative type and quality of the haematoxylin used as a counterstain for IHC may influence the visualisation of ganglion cells. Second, we obtained higher overall numbers of ganglia in CD vs UC and carcinoma patients. This finding may be...
related to differences in tissue-destructive processes, inflammatory responses, and surgical approaches in the three diseases.

The overall distribution of cell counts over the patient groups in our study was comparable with the earlier descriptions by Sokol and Bressenot.\textsuperscript{11,13} We also confirm the previous finding of Sokol that the majority of ganglia in CD patients contained very few inflammatory cells, with only occasionally a more inflamed ganglion being present.\textsuperscript{11} In our opinion, this skewed distribution indicates that correlating the cell count in the most severely inflamed ganglion with disease recurrence risk [as was done in most previous studies] may not necessarily be representative for the overall inflammatory process.

Given the variable conclusions of previous studies on plexitis, we tried to evaluate the validity of the reported criteria based on the cell count data in our cohort. The conclusions of our initial study on plexitis [Ferrante \textit{et al.}]\textsuperscript{10} could not be confirmed in the present cohort, since all our CD patients had at least one inflammatory cell in at least one ganglion of the myenteric plexus. In addition, we identified three separate inflammatory cell types in consecutive sections instead of performing an overall count per ganglion on a single section. The second model [Sokol \textit{et al.}]\textsuperscript{11} could also not be confirmed, since in our study there was no difference of clinical recurrence rates between patients having at least one submucosal ganglion with three mast cells, and patients with less than three mast cells in every submucosal ganglion [30\% vs 47\%, \(p = 0.496\)]. Furthermore, there was also no difference between these two patient groups with regard to the ER rate at 6 months [40\% vs 52\%, \(p = 0.499\)]. Bressenot \textit{et al.} have suggested that either the presence of at least one eosinophil, or more than six lymphocytes in the most severely inflamed ganglion of the submucosal plexus, was independently associated with the risk of surgical recurrence.\textsuperscript{13} In our study group, we could once more not confirm these findings [5\% vs 9\%, \(p = 0.646\) and 0\% vs 7\%, \(p = 1.000\), respectively], possibly because of the low number of patients requiring a second surgical intervention. We also tested the Bressenot model for ER at 6 months and obtained no significant \(p\)-values either [48\% vs 53\%, \(p = 0.639\) and 75\% vs 49\%, \(p = 0.327\), respectively]. Finally, in a recent study, Misteli \textit{et al.} found that severe plexitis [defined as > 10 inflammatory cells/high power

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{A: Actual posterior probabilities of endoscopic recurrence as a function of the percentage non-zero submucosal lymphocyte counts for all our Crohn's disease [CD] study patients. B: Model for posterior probabilities of postoperative endoscopic recurrence [ER] as a function of the percentage non-zero counts and the number of ganglia. Dashed black lines refer to 20, 30, 40, and 50 evaluated ganglia.}
\end{figure}
field] was also associated with surgical recurrence.\textsuperscript{14} We could not reproduce these findings since we did not count inflammatory cells in high power fields in one single slide. However, one should take into account that results of all these retrospective cohort analyses are difficult to compare with our data set, since investigators used different histological techniques [H&E vs IHC], and different endpoints [endoscopic vs clinical vs surgical recurrence].

Our data further showed that only count results for lymphocytes positioned within or apposed to submucosal ganglia were predictive for postoperative ER, and this raises several questions. First, why are lymphocytes more commonly found around submucosal ganglia in the proximal surgical specimens of CD patients than in controls? Second, what attracts them to this position, in other words, what is the inciting stimulus for this type of inflammation? Third, why are the myenteric lymphocyte counts—which are even more elevated than the submucosal counts in CD patients—not predictive for ER? We speculate that lymphocytic inflammation, especially in the superficial part of the submucosal plexus, may be related to increased bacterial translocation through breaches in the mucosal lining. Indeed, we sometimes observed microscopically small, superficial ‘aphthoid’ ulcers or so-called ‘pseudo-pyloric’ metaplasia [interpreted as a sign of healed ulceration] in biopsies from the [grossly] uninvolved proximal margin of our surgical samples. We cannot exclude that similar lesions and the attending inflammation are present beyond the proximal margin, i.e. in the pre-anastomotic region after surgery. Future prospective studies with systematic sampling of per-operative biopsies at some point proximal from the anastomosis could help to solve this issue. Such biopsies may be studied by a variety of morphological and molecular techniques, including gene expression profiling and a search for molecular signatures of mucosal-adherent or tissue-invasive bacteria.

To establish an algorithm for the prediction of ER in actual patients, we performed a post-hoc analysis of the submucosal lymphocyte counts in relation to the risk of ER in our patients. This resulted in a hypothetical posterior probability model which predicts the chance of developing ER based on the percentage non-zero counts. According to this model, a patient with more than 45\% inflammatory ganglia in the submucosal plexus has a greater than 50\% chance of developing ER within 6 months after the operation. One can suppose that the discriminative power of this model would increase when a higher number of ganglia were evaluated. We can confirm this supposition based on our data [see Figure 3B: the curve shows steeper slope when 60 ganglia were scored]. Based on our results, we would recommend: in the pathological examination of Crohn’s ileoresection specimens more extensive sampling of the proximal resection margin, preferably the entire circumference of the bowel; second, ensuring proper orientation of the tissue section at embedding; third, assessment of the actual number of ganglia present in the biopsies on an H&E-stained slide; and finally, applying CD45 immunohistochemistry for the actual analysis of lymphocytes in all the ganglia of the submucosal plexus.

Overall, we have shown that lymphocytes are the most relevant inflammatory cell type with regard to postoperative ER. In a subanalysis on 24 patients, we found that lymphocytes in perivascular tissue in the proximal section margin frequently expressed ITG\(\beta_7\). Since such lymphocytes might drive disease recurrence, blocking lymphocyte trafficking in the postoperative bowel by anti-adhesion molecules may be a promising consideration. Vedolizumab [intravenous anti-\(\alpha_4\beta_7\) integrin antibody] recently showed to be efficacious in patients with moderate to severe Crohn’s disease.\textsuperscript{16} Based on our data, a postoperative prophylactic prevention trial with vedolizumab could be of great interest.

Inevitably, this study had also some limitations. Since we wanted a homogeneous population, patients who received prophylactic therapy or a permanent ileostomy were excluded. Further, patients without any symptoms may have cancelled the postoperative endoscopy after 6 months, which also could have caused a bias. Next, counting dendritic cells was not included in the study. Due to the different subtypes, staining and accurate counting of dendritic cells would include at least three markers, which would not be feasible in daily clinical practice. Finally, we did not look at intra- and inter-observer variability.

In conclusion, we have shown that presence of lymphocytes in the submucosal plexus of the proximal resection margin is associated with an increased probability of postoperative ER in patients undergoing ileocecal resection with ileocolonic anastomosis. The discriminative ability of the proportion of inflamed ganglia increased when more ganglia were evaluated. We therefore advocate extensive sampling of the proximal resection margin with careful attention to embedding procedures.
and combined morphological and IHC evaluation, including CD45 stains. Our study adds further evidence that histological investigation for plexitis is useful to select patients who need more intensive postoperative prophylactic treatment. Since only lymphocytes were predictive for ER in our study, e437 integrin antibodies such as vedolizumab may be particularly useful as postoperative prophylactic treatment.

Funding
None.

Conflicts of Interest
IA received a grant from the Belgian Society of Gastrointestinal Endoscopy; GVA does consultancy for Abbott, BMS, Chiesi, Ferring, Janssen, MSD, Novartis, Shire, Takeda, and Zealand Pharma, receives payment for lectures from Abbott, Apatinis, Ferring, Janssen and MSD, and grants from Abbott, MSD, and Zealand Pharma; SV does consultancy for Abbvie, Ferring, Galapagos, Genentech/Roche, Hospira, Mundipharma, MSD, Shire, and Takeda, and receives payment for lectures from Abbvie, Dr Falk Pharma, Ferring, Hospira, MSD, and Takeda and grants from Abbvie, MSD, and Takeda; GDH does consultancy for Centocor, Galapagos, Genentech, Shire, and Takeda; Marc Ferrante does consultancy for Abbvie, Boehringer-Ingeheil, Ferring, Janssen, Mitsubishi Tanabe, MSD, Takeda, and Zeria, and receives payment for lectures from Chiesi, Falk, Ferring, and Tillotts.

Acknowledgments
This work was supported by the Belgian Society of Gastrointestinal Endoscopy [BSGIE]. GVA, SV, and Marc Ferrante are senior clinical investigators of the Research Foundation – Flanders [FWO], Belgium. We would like to thank Isabelle Terrasson who was the principal study coordinator for this trial.

Author Contributions
BL collected, analysed and interpreted the data and drafted the manuscript. ABO collected the data, critically revised the manuscript for important intellectual content, and approved the final version to be published. IA, XS, GVA, and SV critically revised the manuscript for important intellectual content and approved the final version to be published. AT and KG critically revised the manuscript for important intellectual content, and approved the final version to be published. AT and KG critically revised the manuscript for important intellectual content, and approved the final version to be published. AT and KG critically revised the manuscript for important intellectual content, and approved the final version to be published. AT and KG critically revised the manuscript for important intellectual content, and approved the final version to be published. AT and KG critically revised the manuscript for important intellectual content, and approved the final version to be published.

Supplementary Data
Supplementary data are available at ECCO-JCC online.

References