Detection of Dysplasia or Cancer in 3.5% of Patients with Inflammatory Bowel Disease and Colonic Strictures


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Detection of Dysplasia or Cancer in 3.5% of Patients with Inflammatory Bowel Disease and Colonic Strictures

Short title: Dysplasia and cancer complicating stricture in IBD

Mathurin Fumery1,2, Guillaume Pineton de Chambrun3, Carmen Stefanescu2, Anthony Buisson4, Aude Bressenot5, Laurent Beaugerie6, Aurelien Amiot7, Romain Altwegg8, Guillaume Savoye9, Vered Abitbol10, Guillaume Bouguen11, Marion Simon12, Jean-Pierre Duffas13, Xavier Hébuterne14, Stéphane Nancey15, Xavier Roblin16, Emmanuelle Leturutre17, Gilles Bommelaer4, Jeremie H. Lefevre18, Francesco Brunetti19, Françoise Guillon20, Yoram Bouhnik2, Laurent Peyrin-Biroulet21

1Department of Gastroenterology, Amiens University and Hospital, Université de Picardie Jules Verne, Amiens, France
2Department of Gastroenterology, IBD and Nutrition Support, Beaujon Hospital, University Paris 7 Denis Diderot, Clichy, France
3Department of Gastroenterology, Huriez Hospital, Université Lille Nord de France, Lille, France.
4Department of Hepato-Gastroenterology, University Hospital Estaing of Clermont-Ferrand, Université d' Auvergne, Clermont-Ferrand.
5Department of pathology, University Hospital of Nancy, Vandoeuvre-lès-Nancy, France.
6Department of Gastroenterology, AP-HP, Hôpital Saint-Antoine F-75012; ERL 1057 Inserm/UMRS 7203 and GRC-UPMC 03, UPMC Université Paris 06 F-75005, Paris, France.
7Department of Gastroenterology, Henri Mondor Hospital, UPEC, Creteil, France
8Department of Gastroenterology, Hôpital Saint-Eloi, University Hospital of Montpellier, Montpellier, France
9Department of Gastroenterology, Rouen University and Hospital, Rouen, France
10Department of Gastroenterology, Cochin Hospital, University Paris 5 Descartes, Paris, France.
11Department of Gastroenterology, Pontchaillou Hospital and Rennes University de, Rennes, France.
12Department of Digestive Diseases, Institut Mutualiste Montsouris, Université Paris-Descartes, Paris, France
13Department of Digestive Surgery, Rangueil University Hospital, University of Toulouse, Toulouse, France.
14Department of Gastroenterology and Clinical Nutrition, Nice University Hospital, University of Nice Sophia-Antipolis, Nice.
15Department of Gastroenterology, Hospices Civils de Lyon and University Claude Bernard Lyon 1, Pierre-Benite, France
16Department of Gastroenterology, Saint-Etienne University Hospital, Saint-Etienne, France
17Institute of Pathology, Lille University Hospital, Lille, France
This work was presented in part and orally at the European Crohn’s and Colitis Organization (ECCO) meeting in Copenhagen in 2014, at the Digestive Disease Week (DDW) in Chicago in 2014 and at the UEG week in Vienna in 2014.

**Keywords:** inflammatory bowel disease, colon, stricture, cancer, dysplasia

**Number of words:** 2702

**Guarantor of the article:** Laurent Peyrin-Biroulet, MD, PhD

**Abbreviations:** IBD, inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn’s disease; IBD-U, undetermined inflammatory bowel disease; HGD, High Grade Dysplasia, LGD, Low Grade Dysplasia

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**Competing interests:** None

**Corresponding author:**
Prof. Laurent Peyrin-Biroulet, MD, PhD

Inserm U954 and Department of Hepato-Gastroenterology,
Université de Lorraine,
Allée du Morvan, 54511 Vandoeuvre-lès-Nancy, France
Phone: + 33 3 83 15 36 31 Fax: + 33 3 83 15 36 33
E-mail : peyrinbiroulet@gmail.com


Contributorship statement: MF, data collection, writing of the manuscript, data interpretation; LPB; initiation of study, study supervision, data interpretation, writing of the manuscript; JM, statistical analysis; all the other authors collected the data and critically reviewed the manuscript for intellectual content and approved the article.
Abstract:

**Background & Aims:** Colonic strictures complicate inflammatory bowel disease (IBD) and often lead to surgical resection to prevent dysplasia or cancer. We assessed the frequency of dysplasia and cancer among IBD patients undergoing resection of a colorectal stricture.

**Methods:** We analyzed data from the Groupe d’études et thérapeutiques des affections inflammatoires du tube digestif study. This was a nationwide retrospective study of 12,013 patients with IBD in France who underwent surgery for strictures at 16 centers, from August 1992 through January 2014 (293 patients for a colonic stricture, 248 patients with Crohn’s disease, 51% male, median age at stricture diagnosis was 38 y). Participants had no preoperative evidence of dysplasia or cancer. We collected clinical, endoscopic, surgical, and pathology data and information on outcomes.

**Results:** When patients were diagnosed with strictures, they had IBD for a median time of 8 y (3–14 y). The strictures were a median length of 6 cm (4–10 cm) and caused symptoms in 70% of patients. Of patients with Crohn’s disease, 3 were found to have low-grade dysplasia (1%), 1 was found to have high-grade dysplasia (0.4%), and 2 were found to have cancer (0.8%). Of patients with ulcerative colitis, 1 had low-grade dysplasia (2%), 1 had high-grade dysplasia (2%), and 2 had cancer (5%). All patients with dysplasia or cancer received curative surgery, except 1 who died of colorectal cancer during the follow-up period. No active disease at time of surgery was the only factor associated with dysplasia or cancer at the stricture site (odds ratio, 4.86; 95% confidence interval, 1.11–21.27; P=.036).

**Conclusion:** In a retrospective study of patients with IBD undergoing surgery for colonic strictures, 3.5% were found to have dysplasia or cancer. These findings can be used to guide management of patients with IBD and colonic strictures.

**KEY WORDS:** GETAID study, CD, UC, colon cancer risk factors, carcinogenesis
Inflammatory bowel diseases (IBD) are chronic progressive and destructive conditions (1, 2). Colorectal strictures may occur in both Crohn’s disease (CD) and ulcerative colitis (UC) (3, 4, 5). Old referral centers studies, most of them published before the 90’s, reported a prevalence of colonic stricture in UC ranging from 1.5% to 11% (6, 7, 8, 9, 10, 11) and from 10 to 14% in CD (12, 13). The occurrence of colonic strictures raises concerns about the risk of dysplasia/cancer in IBD patients. The risk of dysplasia or cancer associated with colonic strictures in UC varies among studies and ranged from 0% to 86% (7, 8, 9, 10, 14), while it is poorly known in CD (12). All these studies were characterized by a low number of included patients, the largest one included 52 strictures in UC (9) and 132 in CD patients (12), and little information on clinical and pathological findings levels, none of these studies had detailed information about dysplasia grade or cancer” (7, 8, 9, 12, 14). In two studies, factors with dysplasia or cancer in IBD patients with colonic strictures were appearance late in the course of the disease or at older age, proximal colonic location, and symptomatic strictures (8,12). Diagnosing dysplasia/cancer on endoscopic biopsies is a challenge in clinical practice and the absence of dysplasia or cancer on endoscopic biopsies cannot formally rule out the presence of dysplasia/cancer (10). Hence, the fear of missed colorectal cancer complicating colonic stricture leads frequently to colonic resection in these IBD patients.

The aim of this study was therefore to assess the risk of dysplasia or cancer among IBD patients undergoing intestinal resection for a colorectal stricture without dysplasia or cancer known at the time of surgery in a nationwide retrospective cohort study from the “Groupe d’études et thérapeutiques des affections inflammatoires du tube digestif” (GETAID).
Methods

Identification of cases

All the 42 members of the GETAID, in France, Belgium and Netherlands were invited to participate in this study. Only centers having access to a clinical, surgical or pathological database including all consecutive adult patients operated on for IBD could participate. Inclusion criteria were: (1) adults (≥18 years old) CD, UC or unclassified colitis patients (IBD-U), (2) operated on for colonic stricture, (3) no colonic dysplasia or cancer known at the time of surgery (pre-operative endoscopic colonic biopsies free of dysplasia/cancer). Stricture was defined as a fixed, localized colonic narrowing. Anastomotic strictures and obvious polypoid lesions producing narrowing of the lumen were excluded.

Data collection

Clinical, surgical and pathological data were extracted from patient’s hospital medical records retrospectively, using a standardized questionnaire that was developed specifically for this study. The following clinical data were collected: age, sex, date of IBD diagnosis, familial history of IBD or colorectal cancer, personal history of colorectal cancer/dysplasia or colonic surgery, IBD phenotype and behaviour according to the Montreal classification (15), disease activity at time of surgery based on physicians’ judgment, previous exposure to IBD-related medications (5-aminosalicylate, azathioprine, and anti-TNF). The following information on colonic stricture was collected: date of diagnosis, location, length, symptomatic character, passable with the endoscope. We also collected data on surgery: date of surgery, type of surgery (partial colectomy, total colectomy, coloprotectomy). Presence of dysplasia (low grade or high grade dysplasia) or cancer (histological description) was also recorded.

Statistical analysis

Continuous variables were calculated as medians with interquartile ranges (Q1–Q3). Qualitative variables were given as frequencies and percentages. Comparison of
frequencies was performed using the X2 or Mann Whitney test. Associated factors to dysplasia or cancer were searched by using logistic regression in univariate model. Parameters with p value < 0.1 in univariate analysis were introduced in multivariable regression. Data were analyzed with SAS software V.9.3 (SAS, Chicago, Illinois, USA). Statistical significance was considered p≤0.05.
Results

Study population
By selecting only GETAID centres having a database enrolling consecutive IBD patients, 12,013 IBD patients operated on for IBD in 16 GETAID centres between August 1992 and January 2014 were screened. We identified 293 (2.3%) patients operated on for a colonic stricture with pre-operative endoscopic colonic biopsies free of dysplasia/cancer, including 248 CD, 39 UC, and 6 IBD-U patients (flow chart figure 1). Note that we evaluated in one center the number of patients with a preoperative diagnosis of dysplasia or cancer on colonic stricture. In the center of Amiens, 0.6% of patients operated on for CD and 2.5% of patients operated on for UC were operated on for colonic stricture with a preoperative diagnosis of dysplasia or cancer.

Characteristics of the study population are listed in Table 1. Among the 293 patients operated on for a colonic stricture with pre-operative endoscopic colonic biopsies free of dysplasia/cancer, 51% were males, 42% had CD and 64% UC (CD versus UC; p=0.012). Familial history of IBD was present in 16% of patients, and respectively 4.8% and 9% of patients had a personal or familial history of colonic dysplasia/CRC. CD phenotype according to the Montreal Classification was pure colonic disease (L2) in 56% and ileocolonic disease (L3) in 44% of cases. History of perianal disease was reported in 32% of patients. In UC, 2% of patients had proctitis (E1), 34% left-sided colitis, and 64% pancolitis (E3). 68% of CD patients had a penetrating behaviour associated to strictures. 13% of the overall IBD population experienced a prior colonic resection. Previous exposition to 5-aminosalicylates, azathioprine and anti-TNF were observed in respectively 65%, 68% and 50% of CD patients, and 86%, 48% and 36% of UC patients.

At stricture diagnosis, UC patients (55 [41-65] years) were significantly older than CD (36 [26-48] years) (p=0.003). The median disease duration at stricture diagnosis was 6 [1-12] years in UC and 8 [3-14] years in CD.

Strictures characteristics
Strictures characteristics are detailed in Table 2. All patients underwent preoperative colonoscopy. Strictures were not passable with the endoscope in 64% of cases.
Strictures presented a median length of 6 cm [4-10], and were symptomatic in 70% of patients, with no significant difference between CD and UC. In CD, location of strictures was right colon in 16%, transverse colon in 14%, left colon in 64% and rectum in 6% of patients. In UC, strictures location was right colon in 6%, transverse colon in 13%, left colon in 62%, and rectum in 19% of patients.

**Surgery**

Median disease duration at surgery was 8.8 [4.2-15.7] years without significant difference between CD and UC. Median stricture duration at surgery was shorter in UC (3 [0.6-9.6] months) than in CD 6.3 [1.6-20] months) (p=0.08). Active disease at time of surgery was reported in 87% of CD and 64% of UC patients. Surgical procedure was significantly different between CD and UC (p<0.001): partial colectomy, total colectomy and coloprotectomy were performed respectively in 79%, 19% and 10% of CD patients versus 18%, 28% and 54% of UC patients.

**Risk of dysplasia and cancer complicating colonic strictures**

Dysplasia or cancer was observed in 10 (3.5%) patients with IBD, 6 (2.4%) CD patients, and 4 (10%) UC patients (Table 3). Cancer was observed in 2 (0.8%) CD patients and 2 (5%) UC patients. High-grade dysplasia (HGD) and low-grade dysplasia (LGD) were observed in respectively one (0.4%) and 3 (1.2%) CD patients, and one (2.5%) and one (2.5%) UC patients.

Clinical characteristics of these patients are detailed in Table 4. In UC, all cancer or dysplasia occurred in patients ≥ 59 years, with a long disease duration (≥ 15 years) for all patients. Clinical presentation in CD was different. Dysplasia or cancer occurred in patients with an age ranging from 20 to 70 years, and disease duration ranged between 0 and 32 years. 60% of strictures with dysplasia or cancer were located in descendant colon.

Treatment of cancer was curative in all 4 cases. Three patients with T3N0M0 cancer had only curative surgical resection and one patient with T3N1M0 cancer had both surgical resection and postoperative chemotherapy. After a median follow-up of 4.1 [1.4-7.3] years, one UC patient died of rectal adenocarcinoma, 2.7 years after colectomy with diagnosis of LGD complicating stricture. 3 CD and 1 IBD-U patients
without dysplasia or cancer on surgical resected specimen were diagnosed with cancer after a median time of 11 [10.5-11.5] years and one died of colorectal cancer during follow-up.

**Associated factors to dysplasia or cancer**

In univariate analysis, age at stricture diagnosis (OR, 95%CI; 1.04 [1.004-1.08], p=0.03) and the presence of no active disease at time of surgery (OR, 5.63; [1.43-22.12], p=0.01) were significantly associated to dysplasia or cancer on colonic stricture. In multivariate analysis, only the presence of no active disease at time of surgery was associated to dysplasia or cancer (OR, 4.86; [1.11-21.27], p=0.036).

We evaluated the prevalence of primary sclerosing cholangitis (PSC) in 64 included patients by 4 centers. Among them, only 2 patients had PSC and 2 patients had colonic dysplasia. Among them, one patient had both low-grade dysplasia and PSC. The low prevalence of PSC did not allow us to search for statistical association between PSC and dysplasia/cancer on colonic stricture.
Discussion

The management of IBD patients with colonic stricture(s) remains a challenge in clinical practice. We report here the largest study evaluating the risk of dysplasia or cancer complicating colonic strictures in IBD. More than 12,000 IBD patients operated on for IBD between 1992 and 2014 were screened to identify 293 patients operated on for colonic strictures. Importantly, only strictures without dysplasia or cancer known at the time of surgery were included.

In case of cancer or high-grade dysplasia on colonic stricture, the decision must be the surgical resection (16, 17). In the absence dysplasia or cancer on stricture endoscopic biopsies, the best therapeutic option remains debated. Colonoscopy biopsies cannot rule out dysplasia or cancer on the stricture (10). Rutter et al. had shown that up to 30% of strictures initially considered as benign after endoscopic biopsies, were later discovered to have cancer in the stricture (10). The fear of missed associated colorectal cancer leads frequently to colonic resection. However, therapeutic decisions are taken from data coming from old studies published before the 90’s, which included a low number of patients (7, 8, 9, 10, 12, 14).

Colorectal cancer was observed in 0.8% of CD patients and 5% of UC patients of our cohort of IBD patients. To our knowledge, only one study had evaluated the risk of colorectal cancer complicating stricture in CD (12); 7% (9/132) of patients with colonic stricture had cancer. Five studies had evaluated the risk of cancer complicating colonic strictures in UC; this risk ranged from 0 to 33% (7, 8, 9, 12, 14).

In our study, high grade or low grade dysplasia was observed in 2.5% of UC patients. One study evaluated the risk of dysplasia complicating strictures in UC, which was observed in 11 of 15 (73%) of patients (7).

We observed high grade and low grade dysplasia in respectively 0.4% and 1.2% of CD patients. Hence, the risk of both dysplasia and cancer was lower in CD than in UC. High-grade dysplasia or colorectal adenocarcinoma was observed in 7.5% of resected strictures in UC. Colonic strictures are known to be less frequent in UC than in CD, but their occurrence in UC should systematically raise concerns about cancer.

We also looked at factors associated with dysplasia or cancer on stricture. No active disease at surgery according to physician judgement was the only factor associated
to dysplasia/cancer on stricture identified in multivariate analysis. Factors associated with dysplasia or cancer on colonic strictures remain poorly investigated. Gumaste et al. have identified disease duration, proximal location of stricture and obstructive symptom as associated factors to dysplasia in UC (8). In CD, age at stricture diagnosis was higher in patients with malignant stricture (12).

In our study, both age and disease duration at surgery were significantly associated to dysplasia/cancer in IBD, but only in univariate analysis. All cancer/dysplasia in UC patients developed after more than 15 years of disease evolution and in patients older than 60 years. In CD, clinical presentation of patients with dysplasia/cancer complicating strictures was more heterogeneous. Age at stricture diagnosis ranged from 20 to 70 years in CD, while it ranged from 59 to 65 in UC. Disease duration at stricture diagnosis ranged from 0 to 32 years in CD and from 15 to 30 years in UC. Stricture duration at surgery was shorter in UC than in CD (p=0.08), probably because of the fear of cancer in UC.

In our study, surgical procedure was different between colonic strictures complicating CD or UC. Coloprotectomy was the most frequent surgical procedure in UC while it was partial colectomy in CD patients. International guidelines recommend total colectomy in case of flat high grade dysplasia complicating UC (18). In CD, there are limited data on the appropriate management of dysplasia. Nearly 50% of CD patients who underwent a total coloprotectomy or subtotal colectomy for dysplasia had multifocal dysplasia (19) and more than two thirds who had partial colectomy for dysplasia or cancer developed new dysplasia or cancer over time (20). All these findings suggest that total proctocolectomy should be considered in CD, and probably explain that about 50% of CD patients underwent total colectomy or coloprotectomy in our study.

Only few studies evaluated the outcome and prognosis of colonic cancer complicating stricture. It was previously reported that strictures associated cancer in UC were more aggressive than those do not develop on strictures (8). These results were not confirmed in CD (12). No metastatic cancer was observed in our study and all patients underwent curative treatment. At the end of follow-up, one patient with low-grade dysplasia on stricture died of metachronous colorectal cancer.
There are some limitations of our study. First, it is retrospective. Second, the identification of associated factors should be interpreted cautiously due the relatively low number of dysplasia/cancer. Disease activity, the only factor associated to dysplasia/cancer, was retrospectively and subjectively assessed by the physician. Association between PSC and dysplasia/cancer on colonic stricture could not be assessed. According to the design of the study, these results were only applicable to IBD patients with colonic strictures without dysplasia or cancer on preoperative endoscopic biopsies. However there are several strengths. This nationwide study is currently the largest one having assessed the risk of dysplasia/cancer on colonic stricture in IBD, with the largest number of included strictures. For the first time, only strictures without dysplasia or cancer known at the time of surgery were included. Finally, to reduce bias related to the denominator, only centers having access to a clinical, surgical or pathological database including all consecutive adult patients operated on for IBD could participate in this study.

In conclusion, colorectal strictures represent 2.3% of overall surgery indication in IBD. In patients without dysplasia or cancer known on colorectal stricture at the time of surgery, cancer or dysplasia was observed in 3.5% of the overall IBD population. Cancer was observed in 0.8% of CD and 5% of UC patients. Clinicians have to be aware that negative endoscopic biopsy specimens on stricture do not rule out the presence of dysplasia or cancer in both CD and UC and surgery should be discussed case by case in IBD patients developing colonic stricture, especially in ulcerative colitis.
References


Figure Legends:

Figure 1. Flow chart. IBD, Inflammatory Bowel Disease; CD, Crohn’s disease; UC, Ulcerative colitis.
Table 1. Demographic and clinical characteristics of the study population. CD, Crohn’s disease; UC, Ulcerative colitis.

<table>
<thead>
<tr>
<th></th>
<th>CD (n=248)</th>
<th>UC (n=39)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (%)</td>
<td>42%</td>
<td>64%</td>
<td>0.012</td>
</tr>
<tr>
<td>Median age at stricture diagnosis (years, Q1-Q3)</td>
<td>36 (26-48)</td>
<td>55 (41-65)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Median disease duration at stricture diagnosis (years, Q1-Q3)</td>
<td>8 (3-14)</td>
<td>6 (1-12)</td>
<td>ns</td>
</tr>
<tr>
<td>Disease location at stricture diagnosis</td>
<td>L2 56%</td>
<td>E1 2%</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>L3 44%</td>
<td>E2 34%</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Perineal 32%</td>
<td>E3 64%</td>
<td>ns</td>
</tr>
<tr>
<td>Disease phenotype at stricture diagnosis</td>
<td>B2 32%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B3 68%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active disease at surgery According to physician judgment</td>
<td>87%</td>
<td>64%</td>
<td>ns</td>
</tr>
<tr>
<td>Treatment exposition 5ASA</td>
<td>65%</td>
<td>86%</td>
<td>ns</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>68%</td>
<td>48%</td>
<td>ns</td>
</tr>
<tr>
<td>Anti-TNF</td>
<td>50%</td>
<td>36%</td>
<td>ns</td>
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Table 2. Strictures characteristics. *CD*, Crohn’s *disease*; *UC*, Ulcerative *colitis*.

<table>
<thead>
<tr>
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<th>CD (n=248)</th>
<th>UC (n=39)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length (cm, Q1-Q3)</strong></td>
<td>6 (4-10)</td>
<td>5 (4-10)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Symptomatic (%)</strong></td>
<td>73%</td>
<td>60%</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Passable (%)</strong></td>
<td>33%</td>
<td>38%</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Median stricture duration at surgery (months)</strong></td>
<td>6.3 (1.6-20)</td>
<td>3 (0.6-9.6)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td></td>
<td></td>
<td>p=0.032</td>
</tr>
<tr>
<td>Right colon</td>
<td>16%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Transv. colon</td>
<td>14%</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Left colon</td>
<td>64%</td>
<td>62%</td>
<td></td>
</tr>
<tr>
<td>Rectal</td>
<td>6%</td>
<td>19%</td>
<td></td>
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</table>
Table 3. Risk of low-grade, high-grade dysplasia and cancer in Crohn’s disease and ulcerative colitis. CD, Crohn’s disease; UC, Ulcerative colitis.

<table>
<thead>
<tr>
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<th>CD (n=248)</th>
<th>UC (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low-grade dysplasia (%)</strong></td>
<td>1.2% (3)</td>
<td>2.5% (1)</td>
</tr>
<tr>
<td><strong>High grade dysplasia (%)</strong></td>
<td>0.4% (1)</td>
<td>2.5% (1)</td>
</tr>
<tr>
<td><strong>Cancer (%)</strong></td>
<td>0.8% (2)</td>
<td>5% (2)</td>
</tr>
<tr>
<td><strong>Overall (%)</strong></td>
<td>2.4% (6)</td>
<td>10% (4)</td>
</tr>
</tbody>
</table>
Table 4. Clinical characteristics of patients with dysplasia or cancer complicating strictures. IBD, Inflammatory Bowel Disease; CD, Crohn’s disease; UC, Ulcerative colitis; ADC, Adenocarcinoma; HGD, High grade dysplasia; LGD, Low Grade Dysplasia; ND: missing data

<table>
<thead>
<tr>
<th></th>
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<th>Sex</th>
<th>Age at stricture diagnosis (years)</th>
<th>Disease duration at stricture diagnosis (years)</th>
<th>Symptomatic stricture</th>
<th>Passable stricture in colonoscopy</th>
<th>Localization</th>
<th>Median stricture duration at surgery (months)</th>
<th>Pathological finding</th>
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<td>1</td>
<td>CD</td>
<td>F</td>
<td>53</td>
<td>32</td>
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<td>Left colon</td>
<td>1</td>
<td>ADC</td>
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<tr>
<td>2</td>
<td>CD</td>
<td>F</td>
<td>20</td>
<td>6</td>
<td>No</td>
<td>Yes</td>
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<td>43</td>
<td>ADC</td>
</tr>
<tr>
<td>3</td>
<td>CD</td>
<td>M</td>
<td>59</td>
<td>6</td>
<td>No</td>
<td>Yes</td>
<td>Left colon</td>
<td>43</td>
<td>HGD</td>
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<tr>
<td>4</td>
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<td>M</td>
<td>37</td>
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<td>Yes</td>
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<td>4.4</td>
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<td>CD</td>
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<td>70</td>
<td>30</td>
<td>Yes</td>
<td>No</td>
<td>Left colon</td>
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<tr>
<td>6</td>
<td>CD</td>
<td>F</td>
<td>36</td>
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<td>ND</td>
<td>ND</td>
<td>Rectum</td>
<td>ND</td>
<td>LGD</td>
</tr>
<tr>
<td>7</td>
<td>UC</td>
<td>M</td>
<td>59</td>
<td>23</td>
<td>Yes</td>
<td>Yes</td>
<td>Transverse colon</td>
<td>&lt; 1</td>
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<tr>
<td>8</td>
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<td>60</td>
<td>30</td>
<td>No</td>
<td>No</td>
<td>Left colon</td>
<td>&lt; 1</td>
<td>ADC</td>
</tr>
<tr>
<td>9</td>
<td>UC</td>
<td>M</td>
<td>65</td>
<td>15</td>
<td>No</td>
<td>Yes</td>
<td>Right colon</td>
<td>3</td>
<td>HGD</td>
</tr>
<tr>
<td>10</td>
<td>UC</td>
<td>M</td>
<td>64</td>
<td>16</td>
<td>Yes</td>
<td>Yes</td>
<td>Left colon</td>
<td>&lt; 1</td>
<td>LGD</td>
</tr>
</tbody>
</table>
12,013 patients operated on for IBD

293 patients operated on for colonic strictures without dysplasia or cancer known at the time of surgery

- Crohn’s disease (n=248)
  - Low-grade dysplasia (n=3)
  - High-grade dysplasia (n=1)
  - Cancer (n=2)
- Ulcerative colitis (n=39)
  - Low-grade dysplasia (n=1)
  - High-grade dysplasia (n=1)
  - Cancer (n=2)