Dysplasia in IBD: towards an improved risk stratification

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Dysplasia in IBD: towards an improved risk stratification

Dysplasie bij IBD: richting een verbeterde risicostratificatie

(met een samenvatting in het Nederlands)

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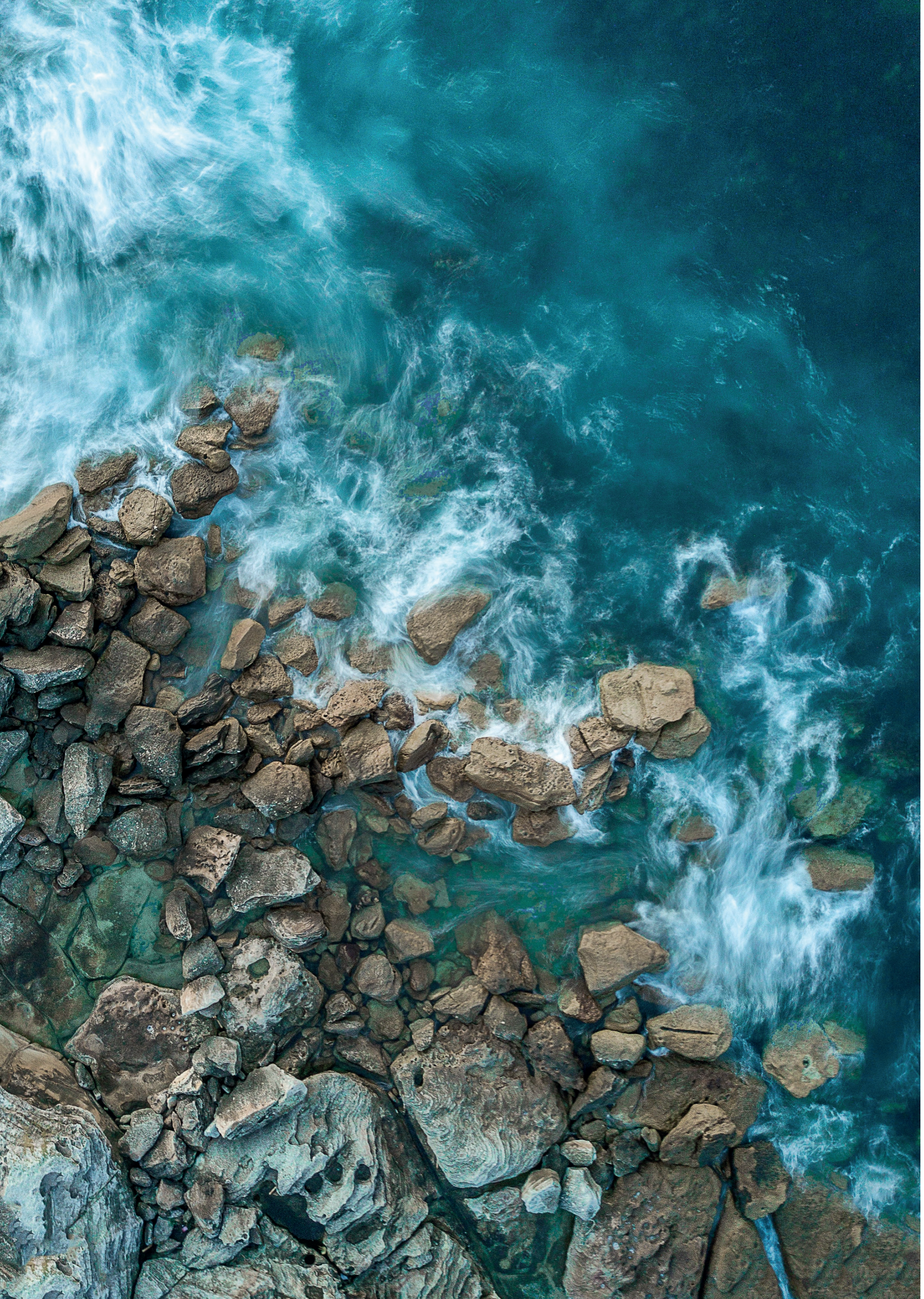

CHAPTER 1

General introduction

Inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), are chronic illnesses characterized by episodes of relapse and remission. For nearly a century, malignant degeneration has been recognized as a lethal complication in patients with IBD involving the colon and research has shown that they are at an overall increased risk of developing colorectal cancer.[1–4] It is generally accepted that the risk of IBD-associated colorectal cancer (CRC) exceeds that of the background population by a factor of 2 and that this increase is comparable between ulcerative colitis and Crohn's colitis.[5,6]

A vast body of research underscores the role of several risk factors modulating this increased risk of CRC. Among these, robust associations have been shown for concurrent PSC, a family diagnosis of CRC, previous dysplasia, total disease duration, chronic disease activity and disease extent.[7–11]

There is a central role for inflammation in carcinogenenesis, which leads to a distinction between the molecular pathways leading up to development of sporadic CRC and colitis-associated CRC.

In sporadic CRC, occurring in the general population, there is a relatively predictable sequence of DNA mutations eventually giving rise to adenoma formation and finally the development of cancer. The timespan of this adenoma-carcinoma sequence is estimated at 7-10 years, based on several large studies.[12,13] Moreover, the fact that adenoma formation precedes cancer development offers a window of opportunity for removal of these precursor lesions. This theory has been tested in a number of studies, laying the groundwork for endoscopic prevention, and leading to a demonstrable reduction in CRC-related mortality.[14]

Patients with long-standing extensive colitis are enrolled in surveillance programs with the aim of detecting colitis-associated dysplasia, premalignant lesions arising from an area of (previous) inflammation. CRC development is thought to occur through a transition of normal mucosa to low-grade dysplasia (LGD) and high-grade dysplasia (HGD), eventually resulting in colitis-associated CRC.[15] Unfortunately, to this date the natural history of neoplasia formation in the colon damaged by IBD (and the

molecular events leading up to it) have not been elucidated, as it is not exactly known in sporadic CRC.[16] The genetic changes causing neoplasia in sporadic and colitis-associated CRC show similarities, but are thought to come up in a different sequence (p53 relatively early, APC relatively late).[17–22] Compared to sporadic adenomas, colitis-associated dysplastic lesions are also macroscopically distinct, more often showing a flat, non-polypoid morphology. Moreover, dysplasia is thought to arise from larger fields of damaged mucosa prone to neoplasia formation (field cancerization) and dysplasia may come up in a multifocal pattern.[23,24]

Over time, colonoscopic surveillance programs have been implemented, using regular intervals in between colonoscopies. The aim of IBD surveillance is the same as that of prevention programs for sporadic CRC; to prevent cancer-related death through early detection of CRC or its precursor lesions (LGD and HGD).

The malignant potential of LGD in colitis shows considerable heterogeneity. Discrete, adenoma-like lesions, even when arising from a background of colitis are thought to have a low risk of progression. More indiscrete, nonpolypoid lesions are thought to have a higher malignant potential. However, there are no clear-cut endoscopic or histologic features that enable us to make a definitive distinction between lesions with either low or high malignant potential. In other words, the natural history of dysplasia in IBD is still rather opaque. A recent meta-analysis showed that among patients with UC and LGD under surveillance, the overall annual risk of CRC after LGD detection was 0.8%.[25] Risk factors for progression were primary sclerosing cholangitis (PSC), invisible dysplasia, distal location, and multifocal LGD.

In addition, important determinants of CRC risk after LGD detection are related to the success of the endoscopic removal of precursor lesions.

Treatment of colitis-associated neoplasia can be either surgical or endoscopic, although in recent years a shift can be seen towards endoscopic management and continued surveillance, in part driven by advances in resection techniques.

IBD surveillance has recently been redefined as an approach that allows the patient to keep their colon as long as possible, with an acceptably low risk of metastatic CRC.

The evidence supporting the effectiveness of IBD surveillance is less robust than that of sporadic CRC prevention programs.

Trials investigating different endoscopic techniques in IBD surveillance largely focus on the sensitivity for dysplasia detection, whereas more advanced outcome measures such as colorectal cancer or mortality are largely absent in these studies. Even though the goal of IBD surveillance is to prevent cancer-related morbidity and death, these outcomes are difficult to study, partly due to a low overall incidence of CRC. For this reason, dysplasia detection is the most commonly used surrogate measure for successful surveillance.

Nonetheless, researchers and clinicians have put great efforts into improving knowledge and developing techniques that are thought to advance IBD surveillance programs. One of the most significant developments has been the introduction of dye-spraying of the colonic mucosa, referred to as chromoendoscopy (CE). CE is an adjunctive technique that has been introduced over the past decade, in order to offer enhanced detection of dysplasia in patients with colitis. It is performed by pancolonic spraying of a blue dye (methylene blue or 0.1–0.5% indigo carmine solutions) on the mucosal surface. In early, preliminary work performed more than 20 years ago, this technique was found to improve the detection of small and flat lesions.[26]

From 2003 onwards, several hallmark trials have shown an increased sensitivity of CE over standard-definition white light endoscopy in detecting dysplasia.[27–29] Following a number of trials confirming these findings, CE has been endorsed by the British Society of Gastroenterology and the European Crohn's and Colitis Organization as the preferred method for IBD surveillance.[30,31] Nonetheless, this technique has not caught on universally, probably due to several factors. First, there has been a concurrent transition from standard-definition (SD) to high-definition (HD) equipment. As of yet, there is no definitive confirmation that CE is also superior to HD white light endoscopy. Second, there may be practical factors that have inhibited the widespread adoption of CE, such as increased cost and time, and lack of training/confidence on the visual interpretation against a blue background.

The SCENIC statement (2015) is one of the most recent position statements on IBD surveillance and addresses a number of areas of contention, specifically focusing on the central role of CE,[32] that had not been previously addressed in the AGA guidelines (2010).[33] In Europe, the ECCO guidelines (2017)[30] are used to guide clinicians in organizing the care with regard to IBD surveillance for their patients. These newer guidelines have recommended to tailor the frequency of surveillance to the level of active inflammation as is seen at the most recent colonoscopy.

Table 1. Overview of the most used guidelines in IBD surveillance

Society	Start surveillance	Risk stratification	Surveillance interval
ECCO (2017)	8 years after onset of symptoms	Stricture or dysplasia within past 5 years, PSC, extensive colitis with severe active inflammation	1 year
		Extensive colitis with mild to moderate active inflammation, post-inflammatory polyps, or first-degree relative with CRC > 50 years	2-3 years
		No intermediate- or high-risk features	5 years
AGA (2010)	8 years after disease onset	Active inflammation, stricture, post-inflammatory polyps, history of dysplasia, first-degree relative with CRC, PSC	1 year
		After 2 negative examinations	1-3 years
ACG (2010)	8-10 years after disease onset	No stratification	1-2years
BSG (2010)	10 years after onset of symptoms	Moderate or severe active inflammation on the previous surveillance examination, stricture or dysplasia within past 5 years, PSC, first-degree relative with CRC <50 years	1 year
		Mild active inflammation on the previous surveillance examination, post-inflammatory polyps, first-degree relative with CRC >50 years	3 years
		No active inflammation on the previous surveillance procedure, left-sided colitis or CD colitis affecting >50% surface area of the colon	5 years

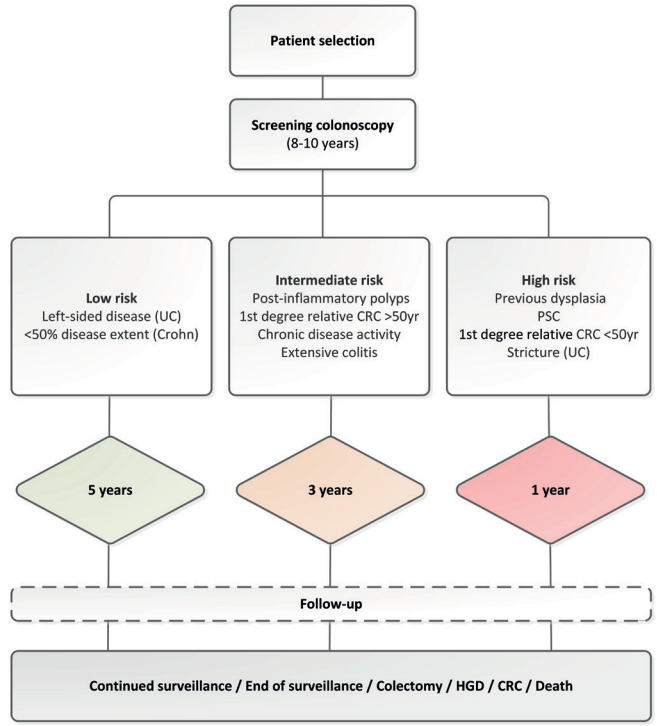

Figure 1. Flowchart displaying the risk groups in the European 3-tier system, with accompanying surveillance intervals

Despite efforts of clinicians and researchers to develop up-to-date and comprehensive guidelines, several findings highlight the still enduring limitations of CRC surveillance programs.

First, although regular endoscopic surveillance is set to start at 8 years after IBD onset, the occurrence of CRC before this time is not an uncommon event, as several studies report CRCs within 8 years after IBD onset.[34–36] Second, despite the fact that patients are undergoing active surveillance, several studies report a continued risk of CRC development while under surveillance with the rate of interval cancers occurring during IBD surveillance to be reported up to 30%.[37] On top of that, these patients are subjected to multiple colonoscopies and hundreds of biopsies in order to prevent CRC. Thus, the current method of surveillance is time-intensive, expensive, burdensome for patients and carries some, albeit small, risk related to biopsies and endoscopic resections.

Aggressive surveillance will most likely result in increased CRC detection, with the risk of medical harm through unnecessary invasive procedures, biopsies and resections. Contrarily, low intensity surveillance may be adequate for selected patients, though it carries a risk of increased interval cancer rates and a false sense of reassurance.

For gastroenterologists managing IBD patients, organizing a surveillance course that is effective in preventing cancer is of utmost importance. However, the tools available for laying out this course are suboptimal. Notably, there is a need for more personalized risk scoring, to ensure the burden of surveillance is proportional to the actual CRC risk.

With surveillance now being routine care for a large number of patients, this thesis aims at filling some of the various knowledge gaps that still exist in this field.

OUTLINE AND AIMS OF THE THESIS

The studies presented in this thesis are both retrospective and prospective, and are based on national and international collaborations between high-volume centers providing specialized IBD care.

The first section of this thesis aims to explore risk factors and provide an adequate definition of the population at risk.

A number of risk factors are known to increase the risk of CRC in IBD patients, one of which is the co-occurrence of primary sclerosing cholangitis (PSC), a chronic liver disease characterized by inflammation and fibrosis of the bile ducts. In **Chapter 2**, we investigated the incidence of colorectal neoplasia in a large cohort in the modern endoscopic era, with a special focus on the IBD-PSC phenotype. To this end, we constructed one of the largest datasets to date by collaborating with the Mt. Sinai Hospital in New York, US. After case ascertainment and dataset harmonization, we were able to generate risk estimations for an IBD surveillance population over a 15-year timeframe, starting in the year 2000. This was followed by estimations of the advanced neoplasia (HGD or CRC) rate after a diagnosis of indefinite for dysplasia (IND) and/or LGD in PSC-IBD patients compared with patients with non-PSC IBD. In

addition, we explored the interaction between active inflammation over time and the risk of neoplasia.

In **Chapter 3**, we assessed the risk of HGD or CRC following the detection of LGD, while distinguishing between LGD lesions detected using either standard-definition WLE, high-definition WLE or chromoendoscopy. We also assessed the risk of advanced neoplasia following the detection of LGD in random biopsies (invisible dysplasia).

While the first chapters focus on high-risk populations, **Chapter 4** zooms in on low-risk IBD patients (without previous neoplasia a family history of CRC or primary sclerosing cholangitis) undergoing endoscopic surveillance. The aim of this study was to investigate whether consecutive surveillance colonoscopies demonstrating no intermediate-risk or high-risk features (such as endoscopic signs of active inflammation or strictures) could predict absent or low rates of advanced colorectal neoplasia during follow-up. For this study, we closely collaborated with both the Mt Sinai Hospital and the University of Manitoba in Winnipeg, Canada.

In **Chapter 5**, the last chapter of the first section, we explored the risk of rectal stump neoplasia and nonmalignant complications in a retrospective cohort of IBD patients who underwent colonic resections. The aim of this study was to assess the incidence rate of dysplasia and cancer in the rectal stump of IBD patients. In addition, we tried to identify risk factors for neoplasia.

Since patients with a diverted rectum may also experience long-term sequelae of colonic surgery, we also aimed to quantify the nonmalignant complications of this procedure and to estimate the quality of life in patients after proctectomy. We did this by performing a prospective, questionnaire-based sub-study.

In the second section of this thesis, we explored new strategies in detection and removal of IBD-associated neoplasia.

The aim of **Chapter 6** was to provide an overview of the current evidence on the use of image-enhanced endoscopy in detecting IBD-associated dysplasia and early-stage CRC. We reviewed the evidence on both dye-spraying image enhancement

(chromoendoscopy) as well as virtual imaging techniques, such as NBI, FICE and i-scan.

The endoscopic equipment and practices in IBD surveillance have changed over time, with an important development being the improved detection of dysplastic lesions. A widespread recommendation is that endoscopists should take additional biopsies from the mucosa surrounding dysplasia, although this recommendation is not based on robust evidence. The aim of **Chapter 7** was therefore to assess the actual dysplasia yield from biopsies taken from the surrounding mucosa in case of dysplastic lesions, employing a retrospective study design.

By using modern techniques, most lesions encountered in IBD patients are visible and can be well delineated. With endoscopic equipment and skills increasing over time, lesions of increasing size can be resected endoscopically. In **Chapter 8**, we specifically looked at large (≥20 mm), non-pedunculated colorectal lesions in patients with IBD and aimed to assess the feasibility and safety of endoscopic resections in these cases.

In **Chapter 9**, we point out knowledge gaps in the surveillance guidelines that are currently unresolved, while providing practical advice on common difficulties for which no recommendations are in place. An additional objective of this chapter is to stimulate further research and propose directions for future studies.

Chapter 10 explores new terrain, in an attempt to develop less invasive measures for screening. Our aim was to find a plasma-based panel of microRNAs that can reliably predict the presence of colorectal neoplasia in IBD patients. From the prospective IBD surveillance cohort, we selected 42 patients for a discovery cohort in which we compared the levels of 758 miRNAs using an OpenArray platform. Next, we selected 40 patients for a validation cohort, in which we assessed the levels of the microRNAs that were differentially expressed in the first part of the study.

REFERENCES

1. Crohn BB, R. H. The sigmoidoscopic picture of chronic ulcerative colitis. *Am J Med Sci.* 220–228 (1925).
2. Eaden, J. A., Abrams, K. R. & Mayberry, J. F. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* **48,** 526–35 (2001).
3. Bernstein, C. N., Blanchard, J. F., Kliewer, E. & Wajda, A. Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* **91,** 854–62 (2001).
4. Jess, T. *et al.* Risk of intestinal cancer in inflammatory bowel disease: a population-based study from olmsted county, Minnesota. *Gastroenterology* **130,** 1039–46 (2006).
5. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
6. Ekbom, A., Helmick, C., Zack, M. & Adami, H. O. Increased risk of large-bowel cancer in Crohn's disease with colonic involvement. *Lancet* **336,** 357–9 (1990).
7. Rutter, M. D. Cancer surveillance in longstanding ulcerative colitis: endoscopic appearances help predict cancer risk. *Gut* **53,** 1813–1816 (2004).
8. Rutter, M. *et al.* Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* **126,** 451–9 (2004).
9. Askling, J. *et al.* Family history as a risk factor for colorectal cancer in inflammatory bowel disease. *Gastroenterology* **120,** 1356–62 (2001).
10. Torres, J., de Chambrun, G. P., Itzkowitz, S., Sachar, D. B. & Colombel, J.-F. Review article: colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **34,** 497–508 (2011).
11. Choi, C.-H. R. *et al.* Forty-Year Analysis of Colonoscopic Surveillance Program for Neoplasia in Ulcerative Colitis: An Updated Overview. *Am. J. Gastroenterol.* **110,** 1022–34 (2015).
12. Zauber, A. G. *et al.* Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N. Engl. J. Med.* **366,** 687–96 (2012).
13. Winawer, S. J. *et al.* Prevention of Colorectal Cancer by Colonoscopic Polypectomy. *N. Engl. J. Med.* **329,** 1977–1981 (1993).
14. Løberg, M. *et al.* Long-term colorectal-cancer mortality after adenoma removal. *N. Engl. J. Med.* **371,** 799–807 (2014).
15. Rutter, M. D. & Riddell, R. H. Colorectal dysplasia in inflammatory bowel disease: a clinicopathologic perspective. *Clin. Gastroenterol. Hepatol.* **12,** 359–67 (2014).

16. Fearon, E. R. & Carethers, J. M. Molecular subtyping of colorectal cancer: time to explore both intertumoral and intratumoral heterogeneity to evaluate patient outcome. *Gastroenterology* **148,** 10–3 (2015).
17. Yin, J. *et al.* p53 point mutations in dysplastic and cancerous ulcerative colitis lesions. *Gastroenterology* **104,** 1633–9 (1993).
18. Brentnall, T. A. *et al.* Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis. *Gastroenterology* **107,** 369–78 (1994).
19. Hao, X. P. *et al.* The spectrum of p53 mutations in colorectal adenomas differs from that in colorectal carcinomas. *Gut* **50,** 834–9 (2002).
20. Baker, A.-M. *et al.* Evolutionary history of human colitis-associated colorectal cancer. *Gut* gutjnl-2018-316191 (2018). doi:10.1136/gutjnl-2018-316191
21. Burmer, G. C. *et al.* Neoplastic progression in ulcerative colitis: histology, DNA content, and loss of a p53 allele. *Gastroenterology* **103,** 1602–10 (1992).
22. Rhodes, J. M. & Campbell, B. J. Inflammation and colorectal cancer: IBD-associated and sporadic cancer compared. *Trends Mol. Med.* **8,** 10–6 (2002).
23. Braakhuis, B. J. M., Tabor, M. P., Kummer, J. A., Leemans, C. R. & Brakenhoff, R. H. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res.* **63,** 1727–30 (2003).
24. Galandiuk, S. *et al.* Field cancerization in the intestinal epithelium of patients with Crohn's ileocolitis. *Gastroenterology* **142,** 855–864.e8 (2012).
25. Fumery, M. *et al.* Incidence, Risk Factors, and Outcomes of Colorectal Cancer in Patients With Ulcerative Colitis With Low-Grade Dysplasia: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* **15,** 665–674.e5 (2017).
26. Jaramillo, E. *et al.* Small, flat colorectal neoplasias in long-standing ulcerative colitis detected by high-resolution electronic video endoscopy. *Gastrointest. Endosc.* **44,** 15–22 (1996).
27. Kiesslich, R. *et al.* Methylene blue-aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology* **124,** 880–8 (2003).
28. Rutter, M. D. Pancolonic indigo carmine dye spraying for the detection of dysplasia in ulcerative colitis. *Gut* **53,** 256–260 (2004).
29. Marion, J. F. *et al.* Chromoendoscopy-targeted biopsies are superior to standard colonoscopic surveillance for detecting dysplasia in inflammatory bowel disease patients: a prospective endoscopic trial. *Am. J. Gastroenterol.* **103,** 2342–9 (2008).

30. Magro, F. *et al.* Third European Evidence-Based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J. Crohn's Colitis* (2017). doi:10.1093/ecco-jcc/jjx008

31. Cairns, S. R. *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* **59,** 666–89 (2010).

32. Laine, L. *et al.* SCENIC International Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease. *Gastroenterology* **148,** 639–651.e28 (2015).

33. Farraye, F. A. *et al.* AGA medical position statement on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* **138,** 738–45 (2010).

34. Cohen-Mekelburg, S. *et al.* Risk of Early Colorectal Cancers Needs to Be Considered in Inflammatory Bowel Disease Care. *Dig. Dis. Sci.* (2019). doi:10.1007/s10620-019-05554-1

35. Hata, K. *et al.* Surveillance Colonoscopy for Ulcerative Colitis-Associated Colorectal Cancer Offers Better Overall Survival in Real-World Surgically Resected Cases. *Am. J. Gastroenterol.* **114,** 483–489 (2019).

36. Lutgens, M. W. M. D. *et al.* High frequency of early colorectal cancer in inflammatory bowel disease. *Gut* **57,** 1246–1251 (2008).

37. Mooiweer, E. *et al.* Incidence of Interval Colorectal Cancer Among Inflammatory Bowel Disease Patients Undergoing Regular Colonoscopic Surveillance. *Clin. Gastroenterol. Hepatol.* **13,** 1656–1661 (2015).

SECTION 1:

Determining the risk of colitis-associated colorectal neoplasia

CHAPTER 2

High risk of advanced colorectal neoplasia in patients with primary sclerosing cholangitis associated with inflammatory bowel disease: results from a multicenter longitudinal surveillance cohort

Clinical Gastroenterology and Hepatology, 2018

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ABSTRACT

Background & Aims: Patients with inflammatory bowel disease (IBD) and primary sclerosing cholangitis (PSC) are at increased risk for colorectal cancer (CRC), but their risk following a diagnosis of low-grade dysplasia (LGD) is not well-described. Our aim was to determine the rate of advanced colorectal neoplasia (aCRN), defined as high-grade dysplasia (HGD) and/or CRC, following a diagnosis of indefinite dysplasia (IND) or LGD in this population.

Methods: Patients with colonic IBD and ≥2 surveillance colonoscopies were identified. The occurrences of IND, LGD, aCRN, and of aCRN following a diagnosis of IND or LGD were compared between PSC-IBD and non-PSC IBD groups. Predictors of aCRN with and without prior LGD were explored.

Results: A total of 1911 patients (293 PSC-IBD, 1618 non-PSC IBD) had 9,265 patient-years (pty) of follow-up. PSC-IBD patients had a 2-fold higher risk of developing aCRN. While mean inflammation scores (0.55 vs. 0.56, p=0.89) and occurrence of LGD (21% vs. 18%, p=0.37) were similar in both groups, the rate of aCRN following a diagnosis of LGD was significantly higher in PSC-IBD versus non-PSC IBD patients (8.4 vs. 3.0 per 100pty, p=0.01). PSC (aHR 2.01, 95%CI: 1.09-3.71), increasing age (aHR 1.03, 95%CI: 1.01-1.05) and active inflammation (aHR 2.39, 95%CI: 1.63-3.49) were independent risk factors for aCRN. Dysplasia was more often endoscopically invisible in PSC-IBD.

Conclusions: PSC remains a strong independent risk factor for aCRN in IBD. Once LGD is detected, aCRN develops at a higher rate and is more often endoscopically invisible in PSC-IBD compared to non-PSC IBD. Our findings add further credence to current recommendations for careful annual colonoscopic surveillance in this high-risk population and consideration of colectomy once LGD is detected.

INTRODUCTION

Patients with inflammatory bowel disease (IBD) are at an increased risk of developing colorectal cancer (CRC).[1,2] The co-occurrence of primary sclerosing cholangitis (PSC)[1-3], a chronic liver disease characterized by progressive inflammation and fibrosis of the bile ducts[4], increases this risk substantially.[5] While an estimated 70% of PSC patients have a concomitant diagnosis of IBD (PSC-IBD)[6], only 3-5% of IBD patients have concomitant PSC, with the diagnosis more common in ulcerative colitis (UC) patients.[7,5] The PSC-IBD phenotype is often characterized by extensive colitis with rectal sparing and backwash ileitis, albeit with a mild and often asymptomatic clinical course.[8–13] However, despite their mild clinical colitis, PSC-IBD patients compared to non-PSC IBD colitis patients have a 3- to 5-fold higher risk of CRC, and the cancers more often occur in the right colon.[14,15] As such, current guidelines recommend that PSC-IBD patients be enrolled in a CRC surveillance program with annual colonoscopy from the time of PSC diagnosis, regardless of their duration of IBD. This is in contrast to those with IBD colitis and no PSC (non-PSC IBD), where CRC surveillance is recommended after 8 years of colonic disease.[5,16–18]

The development of neoplasia in IBD colitis follows a multistep sequence from chronic inflammation and no dysplasia or indefinite dysplasia (IND) to low grade-dysplasia (LGD) and high-grade dysplasia (HGD) prior to final malignant transformation to adenocarcinoma. As such, the presence and grade of dysplasia remain the best current indicators of cancer risk in IBD. There is an increasing tendency to keep patients with LGD on intensive surveillance instead of recommending proctocolectomy.[19,20] However, very few studies have described the risk of aCRN in PSC-IBD patients following a diagnosis of IND and/or LGD.[21,22] Further, the studies that do report on the risk of neoplasia in PSC-IBD patients were performed in an era where imaging enhanced endoscopy and high-resolution endoscopy were not routinely used.

The aims of the present study were to report on the risk of aCRN in a well-characterized cohort of PSC-IBD patients enrolled in a surveillance program in the modern endoscopic era, and to describe the rate of aCRN after a diagnosis of IND

and/or LGD in these patients compared to non-PSC IBD patients with longstanding IBD colitis also undergoing surveillance.

METHODS

Study population and case identification

Patients with established IBD colitis undergoing colonoscopic surveillance between 2000-2015 were retrospectively identified from two databases: a Dutch database inclusive of 2 secondary and 6 tertiary centers and the Mount Sinai Hospital database in New York City inclusive of one tertiary IBD referral center. Cases were identified by query of the electronic health record (EHR)-linked database utilizing both ICD-9 and -10 codes and free text searches for cases of IBD and also free text searches for PSC.

Patient selection (inclusion and exclusion criteria)

After initial identification through the EHR query, individual charts were reviewed. For PSC-IBD patients, a clinical diagnosis of PSC had to be confirmed by distinctive features on cholangiography or liver biopsy (for those with small-duct PSC). Additional inclusion criteria were: 1) diagnosis of IBD (UC, CD, IBD undifferentiated [IBD-U]) with colonic involvement confirmed endoscopically and histologically; 2) confirmed colonic disease duration of at least 8 years for non-PSC IBD or any colonic disease duration for PSC-IBD patients; 3) enrollment in a surveillance program and 4) at least left-sided colitis (UC or IBD-U) or involvement of >30% of the colonic surface (CD or IBD-U). Patients with a history of colectomy prior to enrollment or history of aCRN prior to or at the index colonoscopy during the defined study period were excluded. Surveillance procedures were defined as colonoscopies in which either segmental random biopsies or chromoendoscopy were employed. Colonoscopies with other indications, e.g. medically refractory disease, were excluded. The index colonoscopy was defined as the first surveillance colonoscopy performed within the study period (2000-2015).

Data collection

Database coding was identical for all study populations. The date of study entry was set at the first surveillance colonoscopy in the database. The time of onset of PSC or IBD was determined from EHR review. The date of the last colonoscopy was set as the last day of follow-up.

The following baseline demographic and clinical data were abstracted: date of birth, sex, date of PSC diagnosis (if applicable), date of IBD diagnosis, IBD type, maximum disease extent, and date of prior diagnosis of IND and/or LGD (if applicable). Maximum disease extent was defined as the maximum documented extent of endoscopic disease on any colonoscopy and was coded as follows: extensive/pancolitis (>50%) or intermediate/left-sided (30-50%). Medication exposure (at least one prescription) was recorded for 5-aminosalicylates (5-ASA), thiopurines, and biologics.

Data from each surveillance colonoscopy was recorded, including date of exam, quality of bowel preparation (adequate or inadequate), most proximal extent examined, use of chromoendoscopy, presence and severity of endoscopic inflammation, presence of post-inflammatory polyps ("pseudopolyps"), stricture(s) and visible lesions. Endoscopically detected neoplastic lesions were categorized based on morphology (polypoid/non-polypoid). Endoscopically invisible neoplasia was defined as neoplasia detected in a random biopsy with no corresponding morphologic lesion seen on endoscopy. Right-sided lesions were defined as those proximal to the splenic flexure. Because this was a retrospective study, there was no *a priori* protocol in place to record endoscopic activity in a uniform way. Thus, for each surveillance colonoscopy, severity of active endoscopic inflammation was scored on a 4-point scale for each colonic segment visualized to allow for standardization: 0 (no inflammation/remission), 1 (mild inflammation), 2 (moderate inflammation) or 3 (severe inflammation). A mean inflammatory severity score per patient and per colonoscopy was calculated by dividing the sum of inflammatory severity scores by the total number of colonic segments visualized per colonoscopy and then by the total number of surveillance colonoscopies.

Histology

Dysplasia was recorded as indefinite (IND), low-grade (LGD), or high-grade (HGD). All histologic diagnoses were as reported in the original pathology report; no specimens were re-reviewed or altered for this study. Of note, it is routine clinical practice at each participating institution that all pathology concerning for colorectal neoplasia is reviewed at the time of diagnosis and agreed upon by at least two pathologists.

Primary and secondary outcomes

The primary outcome was a diagnosis of aCRN, defined as HGD or CRC, during follow-up. Secondary outcomes were a diagnosis of IND and/or LGD during follow-up and the development of aCRN following a diagnosis of IND and/or LGD. Factors associated with a diagnosis of aCRN in both PSC-IBD and non-PSC IBD patients with or without a prior diagnosis of IND and/or LGD were explored.

Statistical analysis

Basic descriptive statistics were generated for patients meeting inclusion criteria. Chi-square and Fisher's exact tests were used to compare categorical variables and dichotomous outcomes, while the Student's t-test and Mann-Whitney U-test were used for analyzing continuous data. Incidence rates were calculated as the number of cases per 100 patient-years (pty) of follow-up. Univariate and multivariate Cox-regression modeling was used to identify factors associated with aCRN. The proportional hazards assumption of time-static covariates was assessed using log-log plots and Schoenfeld residuals. Because inflammatory scores were not stable over time, these were inputted as time-changing covariates into the models. Mean inflammation scores were re-calculated at every time-point for each patient to correct for the also variable number of colonoscopies. A p-value of ≤0.10 was used as the cutoff for selecting variables for the multivariate analysis. Kaplan-Meier survival curves were generated to compare cumulative incidence rates. Follow-up data were censored at the last point of colonoscopic follow-up, aCRN diagnosis, or colectomy. All data analyses were performed using SPSS version 22 (Armonk, NY: IBM Corp.)..

Study Oversight

The Institutional Review Board (IRB) for each of the included sites approved the creation and analysis of a longitudinal retrospective cohort database of patients with colonic IBD undergoing surveillance.

RESULTS

Baseline demographic and clinical characteristics

Of 1,911 patients with colonic IBD in the combined database meeting inclusion criteria, 293 patients were confirmed to have PSC-IBD; the remaining 1,618 patients with non-PSC IBD served as the comparison group (Figure 1). Main demographic and clinical features of the cohort are detailed in Table 1. Compared with the non-PSC IBD group, PSC-IBD patients were more often male and younger at study entry, although the age of IBD diagnosis was similar between groups (p=0.11). As expected, UC was the predominant IBD type in the PSC-IBD group. PSC-IBD patients were less frequently exposed to IBD therapy compared to non-PSC IBD patients. In 151 patients (51.5%), the PSC diagnosis was established after the IBD diagnosis, while in 36 (12.3%) PSC was established before the IBD diagnosis. For the remainder, PSC and IBD were diagnosed within the same year or the sequence of diagnoses was not recorded.

The mean follow-up for the total cohort was 4.8 (±3.0) years, with a total of 9,265 patient-years of follow-up; there was no difference in follow-up time between PSC-IBD and non-PSC IBD patients. The number of surveillance colonoscopies performed within the study period was higher in PSC-IBD patients (3.8 vs. 3.3, p<0.01).

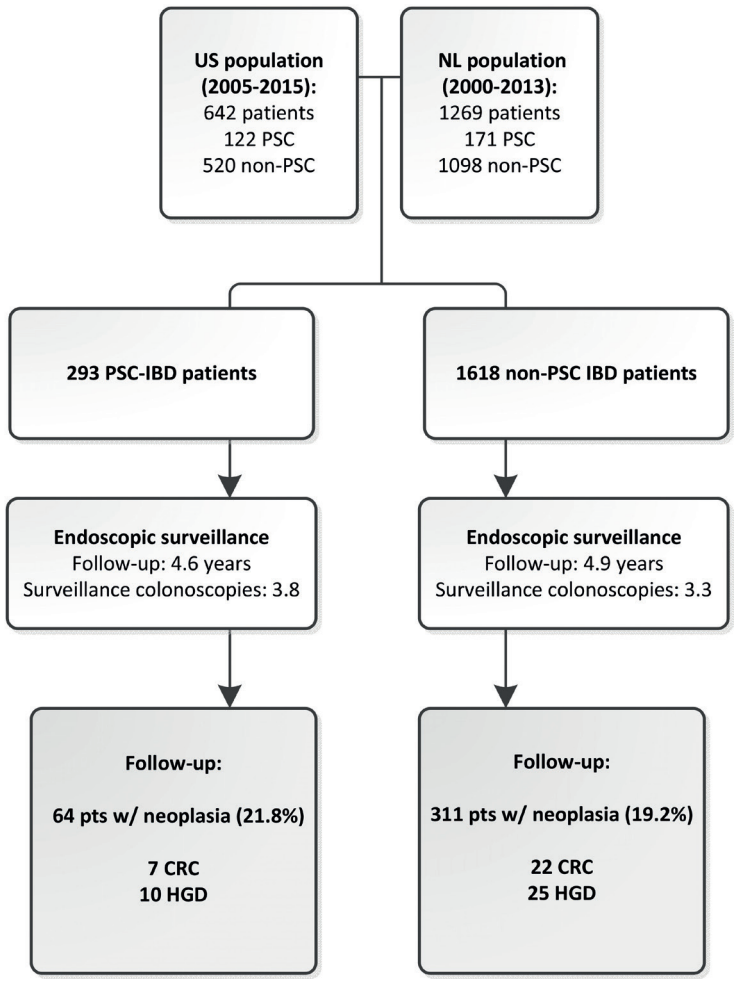


Figure 1 – Description of patient selection and main outcomes in each database. NL, The Netherlands.

Table 1: Baseline characteristics of the study population. PSC: Primary sclerosing cholangitis; IBD: inflammatory bowel disease.

	PSC-IBD (n=293)	Non-PSC IBD (n=1618)	p-value
Male (%)	205 (70.0%)	796 (49.2%)	<0.001
Age at study inclusion, mean (SD)	39 (14)	45 (13)	<0.001
IBD type - Ulcerative colitis - Crohn's colitis - Indeterminate colitis	203 (69.3%) 76 (25.9%) 14 (4.8%)	912 (56.4%) 661 (40.9%) 45 (2.8%)	<0.001
Disease extent - Not specified - Limited extent/proctitis - Intermediate/Left-sided - Extensive/pancolitis	34 (11.8%) 13 (4.5%) 41 (14.2%) 201 (69.6%)	154 (9.6%) 49 (3.1%) 572 (35.8%) 823 (51.5%)	<0.001
Age at IBD diagnosis, mean (SD)	27 (13)	28 (12)	0.11
IBD duration, mean (SD)	12 (10)	17 (9)	<0.001
Age at PSC diagnosis, mean (SD)	32 (14)	-	-
Medication use - 5-ASA - Thiopurines - Biologicals	221 (75.4%) 93 (31.7%) 38 (13.0%)	1316 (81.3%) 825 (51.0%) 402 (24.8%)	0.02 <0.001 <0.001
Duration of follow-up after index colonoscopy - mean (SD) - median	4.6 (3.2) 4.1	4.9 (3.0) 4.5	0.10
Number of surveillance colonoscopies (mean)	3.8	3.3	<0.001

Inflammatory activity

The endoscopic severity of inflammation on surveillance exams was similar between PSC-IBD and non-PSC IBD patients (Supplementary Table 1). The proportion of procedures in which extensive active disease was observed in PSC-IBD vs. non-PSC IBD patients was 27% vs. 12% (p<0.01), 23% vs. 10% (p<0.01), and 27% vs. 10% (p<0.01) for the first, second and third surveillance colonoscopy, respectively. The proportion of patients in endoscopic remission on each of their surveillance colonoscopies during

the entire study period was higher in non-PSC IBD compared to PSC-IBD patients (p=0.02).

Occurrence of aCRN and associated risk factors

Among PSC-IBD patients, aCRN was diagnosed in 17 patients (5.8%), with CRC in 7 (2.4%) and HGD in 10 patients (3.4%) (Table 2). The frequency of aCRN during follow-up was significantly lower in non-PSC IBD patients (2.9%), with CRC and HGD diagnosed in 1.4% and 1.5% patients respectively (p=0.01). The incidence rate of aCRN in PSC-IBD compared to non-PSC IBD patients was significantly higher (1.3 vs. 0.6/100pty, p<0.01) (Figure 2). While aCRN was more often right-sided in PSC-IBD compared to non-PSC IBD patients, this was not statistically significant (53% vs. 31%, p=0.12). Among 40 PSC-IBD patients (14%) where the diagnosis of PSC was newly established within the study period, three cases of aCRN occurred, with a mean duration of 4.0 years (±2.5) between the PSC diagnosis and aCRN occurrence. The primary outcomes stratified by study site are detailed in Supplementary Table 2.

Table 2: Description of the outcomes during the study period. CRC=colorectal cancer, HGD=high-grade dysplasia, LGD=low-grade dysplasia, AN=advanced neoplasia, y=years

	PSC-IBD (n=293)	**Non-PSC IBD (n=1618)**	p-value
Advanced neoplasia (aCRN)	17 (5.8%)	47 (2.9%)	0.01
- CRC	7 (2.4%)	22 (1.4%)	0.19
- HGD	10 (3.4%)	25 (1.5%)	0.03
LGD (patients with ≥1 LGD lesion)	60 (20.5%)	295 (18.2%)	0.37
IND (patients with IND as highest grade lesion)	27 (9.2%)	74 (4.6%)	0.001
Time from IBD diagnosis to aCRN diagnosis, mean (years)	19.4	24.3	0.15
Time from database entry to aCRN diagnosis, mean (years)	4.2	3.4	0.31
Time from LGD to aCRN diagnosis, mean (years)	0.7	1.7	0.12

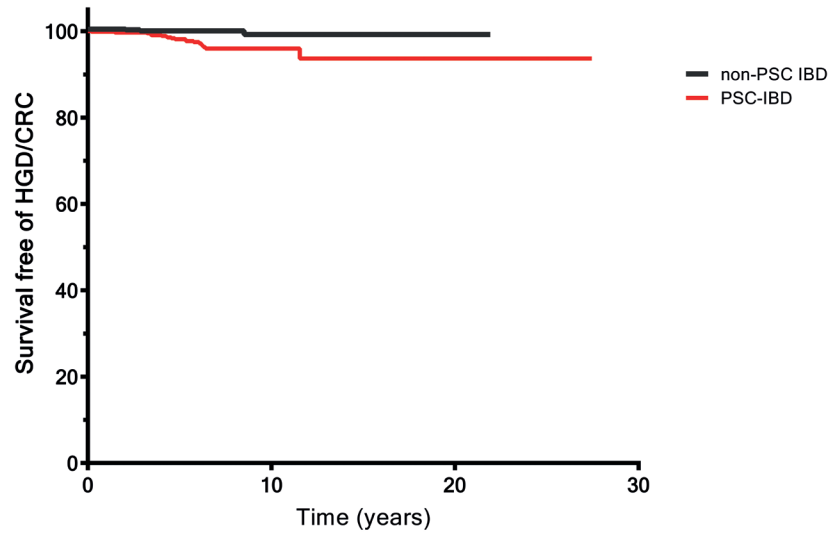


Figure 2 – Kaplan-Meier time-to-event (aCRN) analysis, all patients since study entry.

On multivariate Cox-regression analysis, PSC (adjusted HR (aHR) 2.01, 95%CI: 1.09-3.71), increasing age (aHR 1.03, 95% CI: 1.01-1.05), and active inflammation (aHR 2.39, 95%CI: 1.63-3.49) remained independent predictors of aCRN diagnosis during follow-up (Table 3). Correcting for geography (US vs. Netherlands) did not affect these findings (Supplementary Table 3)

Table 3: Uni- and multivariate Cox-regression analysis for the overall risk of aCRN (all patients).

	Univariate			Multivariate		
Variable	HR	95% CI	P-value	aHR	95% CI	P-value
Age (years)	1.02	1.01-1.04	0.03	1.03	1.01-1.05	0.007
Age at IBD diagnosis	1.00	0.98-1.02	0.78			
Sex (male)	1.83	1.08-3.08	0.02	1.62	1.94-2.79	0.08
PSC	2.13	1.22-3.70	0.01	2.01	1.09-3.71	0.03
Inflammation severity, mean [0-3]*	2.14	1.48-3.09	<0.001	2.39	1.63-3.49	<0.001
IBD type (reference: UC)	0.99	0.60-1.61	0.95			
Maximum disease extent (reference: pancolitis)	1.43	0.85-2.41	0.18			
Thiopurine exposure	0.84	0.51-1.40	0.85			
Biological exposure	0.72	0.36-1.46	0.36			
5-ASA exposure	1.14	0.58-2.25	0.70			
Number of surveillance procedures	0.96	0.84-1.09	0.53			

*entered as time-changing covariate; 0 (no inflammation/remission), 1 (mild), 2 (moderate), 3 (severe)

Risk of aCRN following a diagnosis of IND and/or LGD

The number of patients in the total cohort with at least one diagnosis of IND was 147 (7.7%). In 101 patients (5.3%) no additional dysplasia was detected. Among patients with a diagnosis of IND, the rate of developing aCRN following detection of IND was higher in PSC-IBD compared to non-PSC IBD patients (p=0.02, Supplementary Figure 1). However, when patients with a synchronous or metachronous diagnosis of LGD (n=46) were excluded from this analysis (i.e. no grade of dysplasia higher than IND), this difference was no longer significant.

The occurrence of at least one LGD-containing lesion during the study period was similar for both PSC-IBD and non-PSC IBD patients (21% vs. 18%, p=0.37). Despite a similar proportion of patients with LGD, the rate of developing aCRN following detection of LGD was almost 3-fold higher in PSC-IBD compared to non-PSC IBD patients (8.4 vs. 3.0/100pty, p=0.01, Figure 3).

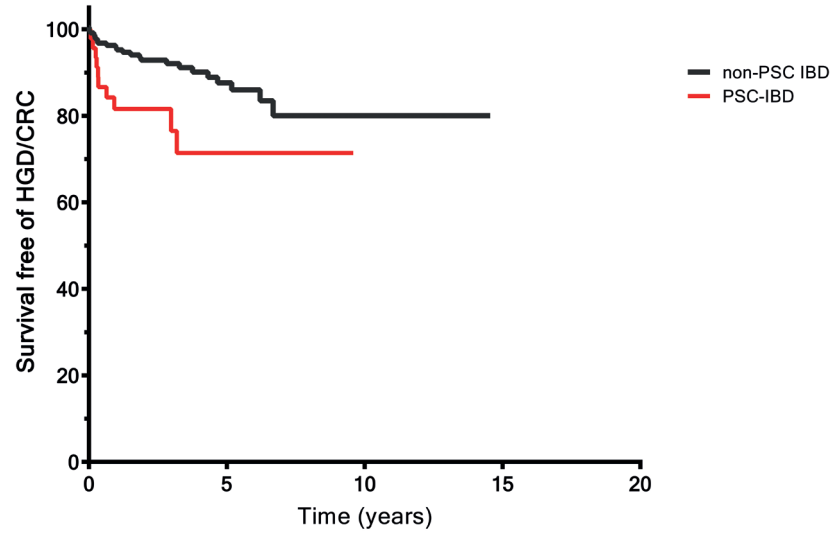


Figure 3 – Kaplan-Meier time-to-event (aCRN) analysis, patients with LGD only.

For the subgroup of patients with LGD, the number of patients in whom endoscopically invisible LGD was detected over the course of surveillance was higher in PSC-IBD patients (38% vs. 22%, p=0.01). The proportion of invisible LGD cases among the total number of LGD cases (per-colonoscopy analysis) was also higher. In a sub-analysis of the Netherlands population, we corrected for the total number of random biopsies taken (107,745 biopsies in total); the number of random biopsies needed to detect invisible dysplasia was 826 in PSC-IBD patients compared to 1,703 in non-PSC IBD patients.

On univariate Cox regression analysis, only PSC and multifocal dysplasia were associated with higher risk of aCRN diagnosis following LGD detection, while polypoid morphology of the lesion (vs. nonpolypoid or invisible) was associated with a lower risk. On multivariate analysis, only polypoid morphology remained significant and was associated with a reduced risk of aCRN (aHR 0.31, 95%CI: 0.14-0.65) following LGD detection compared to nonpolypoid or endoscopically invisible lesions (Supplementary Table 4).

DISCUSSION

In this large, multicenter, cross-national longitudinal cohort study of patients with confirmed IBD colitis undergoing colonoscopic CRC surveillance, we report a higher risk of aCRN in patients with concomitant PSC as compared to those without PSC, in the current era of improved endoscopic technology and more effective medical therapy for inflammation. While these findings corroborate previous studies, we further expand knowledge in the field by reporting an even higher risk of aCRN following detection of LGD (but not IND alone). That LGD was more often endoscopically invisible in PSC-IBD compared to non-PSC IBD justifies the more intensive management considerations for this population. Our findings suggest that while continued meticulous CRC surveillance with annual colonoscopy is indicated in the absence of dysplasia for PSC-IBD patients, the detection of LGD or higher-grade pathology should lead to a careful weighting of the pros and cons of more aggressive therapeutic management, including colectomy.

In our well-characterized surveillance cohort, which to our knowledge is the largest published cohort of PSC-IBD patients undergoing surveillance, we found that PSC-IBD is associated with a 2-fold higher risk of aCRN. This risk is slightly lower than prior studies and a recent meta-analysis of 16 studies that reported a 3.4 fold higher odds for colorectal neoplasia in PSC-IBD patients.[23] Importantly, the increased risk in our study remained after correcting for active endoscopic inflammation over time (which was employed as a time-changing covariate rather than a mean overall score). Endoscopic activity assessed during subsequent colonoscopies was strongly associated with the risk of future aCRN, congruent with studies in the non-PSC IBD population.[24–26] Whether the outcomes of endoscopic inflammation compared to histologic inflammation are distinct remains a question for future investigation.

The increased risk of CRC in patients with PSC and concomitant colonic IBD has firmly been established, although the underlying mechanisms remain unclear.[3,27–30] The nearly 3-fold higher rate of aCRN (HGD and/or CRC) diagnosis following LGD detection, as well as the difference in location, morphology, and endoscopic conspicuousness of dysplasia in PSC-IBD compared to non-PSC IBD suggests

nuances in the pathogenesis of neoplasia between these groups. Several authors have proposed a role for altered colonic bile composition in carcinogenesis. A right-sided predominance of neoplasia reinforces this hypothesis, as well as several studies directly measuring the bile acid composition in both animals and humans.[29,31,32] There is also evidence supporting the notion that PSC patients have an altered colonic microbiome irrespective of concurrent IBD or UDCA treatment.[33] Still, whether these bacterial alterations are a cause or a consequence of the disease characteristics specific to PSC remains to be clarified. PSC patients also share a distinct genotype[34], which may further predispose them to neoplastic progression. More likely, the underlying etiologies are multifactorial with roles for gene-environment interactions, the microbiome, and epigenetic modifications. Further investigations will hopefully open new avenues for novel therapeutic discovery and primary and secondary prevention.

All told, because the mechanisms underlying PSC as an independent risk factor for CRC in the setting of IBD colitis are unclear, the best strategy for CRC prevention in PSC-IBD remains frequent, attentive surveillance colonoscopy. An important observation from our study that distinguishes PSC-IBD from non-PSC IBD patients, is that dysplasia was more often detected in random biopsies. While previous retrospective studies have shown a low overall yield for dysplasia with random biopsies as opposed to only targeted biopsies of visible lesions, there was higher yield for dysplasia on random biopsy in those with concurrent PSC.[35,36] Our data further add to this body of evidence, and it can therefore be questioned whether the current recommendation, based on the results of prospective studies, to move away from random biopsies as part of CRC surveillance should preclude PSC-IBD patients.[37,38] During surveillance examinations, particular attention should be paid to the proximal colon as right-sided cancers seem to be more common in PSC-IBD compared to non-PSC IBD colitis.[32] While the proportion of right-sided aCRN was higher in the PSC- subgroup, this difference was not statistically significant in the present study and may be due to insufficient power; it may also reflect selection bias since one of our inclusion criteria for the non-PSC IBD subgroup was at least left-sided disease

extent or more than 30% involvement, and thus may not represent the overall IBD population. Our study confirms that the date of PSC diagnosis is particularly relevant when risk-stratifying patients, since it seems that the risk of neoplastic progression is highest within the first few years of the PSC diagnosis.[39] Thus, while CRC surveillance is recommended after disease duration of 8 years in patients with colonic IBD and no PSC[40], CRC surveillance at the time of diagnosis in the setting of PSC is recommended and further corroborated by our findings.

Our study has several strengths. In addition to being perhaps the largest IBD surveillance cohort in the modern era, our cohort is particularly robust since each patient was confirmed to have colonic IBD and to be actively enrolled in a colonoscopic CRC surveillance program. Comprehensive data on disease history and endoscopic findings during surveillance allowed for more accurate neoplastic risk assessment, particularly with respect to measurement of inflammatory burden over time. Importantly, detailed information on inflammatory activity at each colonoscopy was incorporated into the analysis for more accurate assessment of aCRN development in PSC-IBD.

Our study also has some limitations, most notably the retrospective design. Despite the large size of our PSC-IBD cohort, additional sub-analyses, such as stratification according to IBD type or medication use, yielded insufficient power to permit meaningful conclusions. Although we combined surveillance cohorts from two different countries, we predefined the inclusion/exclusion criteria, variables to be assessed, and definitions of outcomes. Combining these two cohorts enhanced not only our power to detect meaningful differences, but also the generalizability of our findings given that our study population included patients from affiliated community-based sites as well as tertiary IBD referral centers. That said, there may be unmeasured differences in care pathways between the Netherlands and US leading to heterogeneity in our study results. It is important to note, however that after adjusting for study site and clinico-demographic differences between the Netherlands and US cohorts, our results remained significant (supplementary table 3). The lack of standardized guidelines for the use of chromoendoscopy for CRC

surveillance in IBD colitis unfortunately precluded a meaningful analysis of its impact on dysplasia detection since ≤10% of exams were performed with chromoendoscopy. Finally, while no samples were re-reviewed by pathologists for the purposes of this study, it is routine practice at all institutions participating in this study that whenever there is a diagnosis of CRN, that the specimen is reviewed by two pathologists and consensus reached before final reporting.

In summary, using a large well-characterized cohort of patients with confirmed colonic IBD undergoing surveillance between 2000-2015, we substantiated prior smaller reports of the increased risk of aCRN in patients with concurrent PSC-IBD compared to non-PSC IBD patients undergoing surveillance. Novel findings of our study include the significantly higher rate of aCRN diagnosis following a diagnosis of LGD in the setting of PSC complicating IBD. This finding together with a higher risk of invisible dysplasia in PSC-IBD highlights the need for an ongoing strict CRC surveillance program in these patients and a low threshold to advise colectomy once LGD is detected in this select population.

REFERENCES

1. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
2. Jess, T., Rungoe, C. & Peyrin–Biroulet, L. Risk of Colorectal Cancer in Patients With Ulcerative Colitis: A Meta-analysis of Population-Based Cohort Studies. *Clin. Gastroenterol. Hepatol.* **10,** 639–645 (2012).
3. Claessen, M. M. H., Vleggaar, F. P., Tytgat, K. M. A. J., Siersema, P. D. & van Buuren, H. R. High lifetime risk of cancer in primary sclerosing cholangitis. *J. Hepatol.* **50,** 158–64 (2009).
4. Horsley-Silva, J. L., Carey, E. J. & Lindor, K. D. Advances in primary sclerosing cholangitis. *Lancet Gastroenterol. Hepatol.* **1,** 68–77 (2016).
5. Torres, J., Pineton de Chambrun, G., Itzkowitz, S., Sachar, D. B. & Colombel, J.-F. Review article: colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **34,** 497–508 (2011).
6. Weismüller, T. J. *et al.* Patient Age, Sex, and Inflammatory Bowel Disease Phenotype Associate With Course of Primary Sclerosing Cholangitis. *Gastroenterology* (2017). doi:10.1053/j.gastro.2017.02.038
7. Olsson, R. *et al.* Prevalence of primary sclerosing cholangitis in patients with ulcerative colitis. *Gastroenterology* **100,** 1319–23 (1991).
8. Boonstra, K. *et al.* Primary sclerosing cholangitis is associated with a distinct phenotype of inflammatory bowel disease. *Inflamm. Bowel Dis.* **18,** 2270–2276 (2012).
9. O'Toole, A. *et al.* Primary Sclerosing Cholangitis and Disease Distribution in Inflammatory Bowel Disease. *Clin. Gastroenterol. Hepatol.* **10,** 439–441 (2012).
10. Penna, C. *et al.* Pouchitis after ileal pouch-anal anastomosis for ulcerative colitis occurs with increased frequency in patients with associated primary sclerosing cholangitis. *Gut* **38,** 234–9 (1996).
11. Loftus, E. V *et al.* PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* **54,** 91–96 (2005).
12. de Vries, A. B., Janse, M., Blokzijl, H. & Weersma, R. K. Distinctive inflammatory bowel disease phenotype in primary sclerosing cholangitis. *World J. Gastroenterol.* **21,** 1956–71 (2015).
13. Sinakos, E. *et al.* Inflammatory Bowel Disease in Primary Sclerosing Cholangitis. *Inflamm. Bowel Dis.* **19,** 1004–1009 (2013).
14. Lundqvist, K. & Broomé, U. Differences in colonic disease activity in patients with ulcerative colitis with and without primary sclerosing cholangitis: a case control study. *Dis. Colon Rectum* **40,** 451–6 (1997).

15. Soetikno, R. M., Lin, O. S., Heidenreich, P. A., Young, H. S. & Blackstone, M. O. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. *Gastrointest. Endosc.* **56,** 48–54 (2002).
16. Farraye, F. A. *et al.* AGA medical position statement on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* **138,** 738–45 (2010).
17. Annese, V. *et al.* European evidence based consensus for endoscopy in inflammatory bowel disease. *J. Crohns. Colitis* **7,** 982–1018 (2013).
18. Broomé, U., Löfberg, R., Lundqvist, K. & Veress, B. Subclinical time span of inflammatory bowel disease in patients with primary sclerosing cholangitis. *Dis. Colon Rectum* **38,** 1301–5 (1995).
19. Pekow, J. R. *et al.* Outcome after surveillance of low-grade and indefinite dysplasia in patients with ulcerative colitis. *Inflamm. Bowel Dis.* **16,** 1352–1356 (2010).
20. Fumery, M. *et al.* Incidence, Risk Factors, and Outcomes of Colorectal Cancer in Patients with Ulcerative Colitis with Low-Grade Dysplasia: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* (2016). doi:10.1016/j.cgh.2016.11.025
21. Venkatesh, P. G. K., Jegadeesan, R., Gutierrez, N. G., Sanaka, M. R. & Navaneethan, U. Natural history of low grade dysplasia in patients with primary sclerosing cholangitis and ulcerative colitis. *J. Crohn's Colitis* **7,** 968–973 (2013).
22. Eaton, J. E. *et al.* The fate of indefinite and low-grade dysplasia in ulcerative colitis and primary sclerosing cholangitis colitis before and after liver transplantation. *Aliment. Pharmacol. Ther.* **38,** 977–87 (2013).
23. Zheng, H.-H. & Jiang, X.-L. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease. *Eur. J. Gastroenterol. Hepatol.* **28,** 1 (2016).
24. Rutter, M. *et al.* Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* **126,** 451–9 (2004).
25. Rubin, D. T. *et al.* Inflammation is an independent risk factor for colonic neoplasia in patients with ulcerative colitis: a case-control study. *Clin. Gastroenterol. Hepatol.* **11,** 1601-8–4 (2013).
26. Gupta, R. B. *et al.* Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* **133,** 1099-105–1 (2007).
27. Broomé, U., Löfberg, R., Veress, B. & Eriksson, L. S. Primary sclerosing cholangitis and ulcerative colitis: evidence for increased neoplastic potential. *Hepatology* **22,** 1404–8 (1995).
28. Kornfeld, D., Ekbom, A. & Ihre, T. Is there an excess risk for colorectal cancer in patients with ulcerative colitis and concomitant primary sclerosing cholangitis? A population based study. *Gut* **41,** 522–5 (1997).

29. Shetty, K., Rybicki, L., Brzezinski, A., Carey, W. D. & Lashner, B. A. The risk for cancer or dysplasia in ulcerative colitis patients with primary sclerosing cholangitis. *Am. J. Gastroenterol.* **94,** 1643–9 (1999).

30. Navaneethan, U. *et al.* Temporal trends in colon neoplasms in patients with primary sclerosing cholangitis and ulcerative colitis. *J. Crohn's Colitis* **6,** 845–851 (2012).

31. Barrasa, J. I., Olmo, N., Lizarbe, M. A. & Turnay, J. Bile acids in the colon, from healthy to cytotoxic molecules. *Toxicol. In Vitro* **27,** 964–77 (2013).

32. Claessen, M. M. H. *et al.* More right-sided IBD-associated colorectal cancer in patients with primary sclerosing cholangitis. *Inflamm. Bowel Dis.* **15,** 1331–6 (2009).

33. Sabino, J. *et al.* Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. *Gut* **65,** 1681–1689 (2016).

34. Ji, S.-G. *et al.* Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease. *Nat. Genet.* (2016). doi:10.1038/ng.3745

35. Navaneethan, U. *et al.* Random biopsies during surveillance colonoscopy increase dysplasia detection in patients with primary sclerosing cholangitis and ulcerative colitis. *J. Crohns. Colitis* **7,** 974–81 (2013).

36. van den Broek, F. J. C. *et al.* Random biopsies taken during colonoscopic surveillance of patients with longstanding ulcerative colitis: low yield and absence of clinical consequences. *Am. J. Gastroenterol.* **109,** 715–22 (2014).

37. Moussata, D. *et al.* Are random biopsies still useful for the detection of neoplasia in patients with IBD undergoing surveillance colonoscopy with chromoendoscopy? *Gut* gutjnl-2016-311892 (2017). doi:10.1136/GUTJNL-2016-311892

38. Watanabe, T. *et al.* Comparison of Targeted vs Random Biopsies for Surveillance of Ulcerative Colitis-associated Colorectal Cancer. *Gastroenterology* (2016). doi:10.1053/j.gastro.2016.08.002

39. Navaneethan, U. *et al.* Duration and severity of primary sclerosing cholangitis is not associated with risk of neoplastic changes in the colon in patients with ulcerative colitis. *Gastrointest. Endosc.* **75,** 1045–1054.e1 (2012).

40. Lutgens, M. W. M. D. *et al.* High frequency of early colorectal cancer in inflammatory bowel disease. *Gut* **57,** 1246–1251 (2008).

SUPPLEMENTARY MATERIALS

Supplementary Table 1. Inflammatory parameters during surveillance. *=corrected for total number ofw surveillance colonoscopies per patient

	PSC-IBD	Non-PSC IBD	p-value
Severity of active inflammation, mean [0-3]*	0.55	0.56	0.89
Extent of active inflammation, mean [0-3]*	1.36	1.17	0.003
Activity ratio for all surveillance colonoscopies (active:inactive)	45%	41%	0.19
No inflammation on ALL surveillance colonoscopies	76 (27.1%)	546 (34.1%)	0.02
Inflammation extent (1st colonoscopy) - No activity - Limited - Intermediate - Extensive/pancolitis	127 (53.6%) 7 (3.0%) 38 (16.0%) 65 (27.4%)	864 (57.9%) 89 (6.0%) 363 (24.3%) 176 (11.8%)	0.001
Inflammation extent (2nd colonoscopy) - No activity - Limited - Intermediate - Extensive/pancolitis	125 (55.3%) 9 (4.0%) 40 (17.7%) 52 (23.0%)	866 (61.2%) 109 (7.7%) 297 (21.0%) 141 (10.0%)	<0.001
Inflammation extent (3rd colonoscopy) - No activity - Limited - Intermediate - Extensive/pancolitis	102 (57.3%) 6 (3.4%) 22 (12.4%) 48 (27.0%)	584 (63.6%) 79 (8.6%) 164 (17.9%) 92 (9.9%)	<0.001
Endoscopic inflammation severity (1st colonoscopy) - No activity - Mild - Moderate - Severe	160 (57.1%) 100 (35.7%) 19 (6.8%) 1 (0.4%)	924 (57.8%) 495 (30.9%) 131 (8.2%) 50 (3.1%)	0.20

Table S1. Continued

	PSC-IBD	Non-PSC IBD	p-value
Endoscopic inflammation severity (2nd colonoscopy) - No activity - Mild - Moderate - Severe	125 (53.6%) 89 (38.2%) 16 (6.9%) 3 (1.3%)	864 (59.3%) 445 (30.5%) 101 (6.9%) 48 (3.3%)	0.77
Endoscopic inflammation severity (3rd colonoscopy) - No activity - Mild - Moderate - Severe	102 (55.4%) 63 (34.2%) 13 (7.1%) 6 (3.3%)	583 (61.6%) 276 (29.2%) 64 (6.8%) 23 (2.4%)	0.17

Supplementary Table 2. Database characteristics: comparison Netherlands (NL)-United States (US)

	NL (n=1269)	US (n=642)	p-value
Male (%)	674 (53.1%)	327 (50.9%)	0.37
Age at study inclusion, mean (Standard deviation [SD])	45 (12)	41 (15)	<0.001
Age at IBD diagnosis, mean (SD)	29 (12)	26 (14)	<0.001
Primary sclerosing cholangitis (PSC)	171 (13.5%)	122 (19.0%)	0.002
Age at PSC diagnosis, mean (SD)	33 (12)	32 (16)	0.37
IBD type - Ulcerative colitis - Crohn's colitis - Indeterminate colitis	800 (63.0%) 434 (34.2%) 35 (2.8%)	315 (49.1%) 303 (47.2%) 24 (3.7%)	<0.001
Extensive disease / pancolitis	686 (54.1%)	338 (52.6%)	0.56
Medication use - 5-Aminosalicylates - Thiopurines - Biologicals	999 (78.7%) 556 (43.8%) 156 (12.3%)	543 (83.8%) 362 (56.4%) 284 (44.2%)	0.008 <0.001 <0.001
Number of surveillance colonoscopies (mean)	3.4	3.3	0.25
Interval between surveillance colonoscopies, years (mean)	1.6	1.2	<0.001

Table S2. Continued

	NL (n=1269)	US (n=642)	p-value
Neoplasia Outcomes:			
Colorectal cancer (CRC)	17 (1.3%)	12 (1.9%)	0.37
High-grade dysplasia (HGD)	15 (1.2%)	20 (3.1%)	0.003
Low-grade dysplasia (LGD)	264 (20.8%)	88 (13.7%)	<0.001

Supplementary Table 3. Uni- and multivariate Cox-regression analysis for the overall risk of aCRN* (all patients), corrected for study site.

	Univariate			Multivariate		
Variable	HR	95% CI	p-value	aHR	95% CI	p-value
PSC	2.13	1.22-3.70	0.008	1.85	1.00-3.43	0.049
Inflammation (severity [0-3])**	2.14	1.48-3.09	<0.001	2.08	1.42-3.07	<0.001
Sex (reference: male)	1.83	1.08-3.08	0.02	1.68	0.97-2.89	0.06
IBD type (reference: UC)	0.99	0.60-1.61	0.95			
Maximum disease extent (reference: pancolitis)	1.43	0.85-2.41	0.18			
Age at IBD diagnosis	1.00	0.98-1.02	0.78			
Age (years)	1.02	1.01-1.04	0.03	1.03	1.01-1.05	0.004
Thiopurine exposure	0.84	0.51-1.40	0.85			
Biological exposure	0.72	0.36-1.46	0.36			
5-aminosalicylate exposure	1.14	0.58-2.25	0.70			
Number of surveillance procedures	0.96	0.84-1.09	0.53			
Population (reference: US)	2.82	1.72-4.62	<0.001	2.20	1.30-3.74	0.003

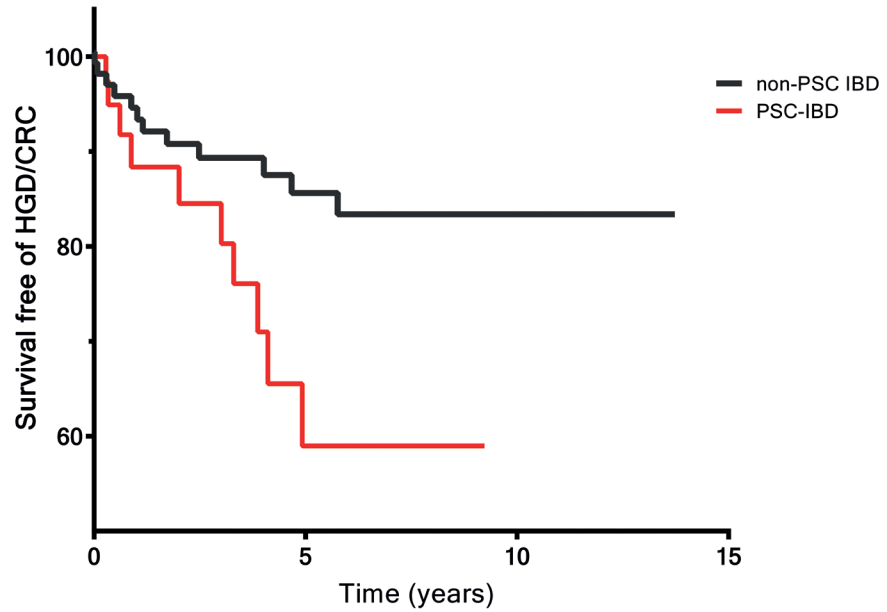
*advanced colorectal neoplasia, aCRN: defined as colorectal cancer and/or high-grade dysplasia

**entered as time-changing covariate; 0 (no inflammation/remission), 1 (mild), 2 (moderate), 3 (severe)

Supplementary Table 4. Uni- and multivariate Cox-regression analysis for the risk of aCRN* following detection of low-grade dysplasia (LGD).

	Univariate			Multivariate		
Variable	HR	95% CI	p-value	aHR	95% CI	p-value
PSC	2.52	1.19-5.31	0.02	1.79	0.83-3.88	0.14
Sex (reference: male)	1.23	0.60-2.49	0.57			
Thiopurine exposure	1.20	0.60-2.40	0.60			
Biological exposure	0.74	0.23-2.44	0.74			
5-Aminosalicylate exposure	1.07	0.44-2.59	0.88			
Dysplasia characteristics						
Distal location	1.69	0.77-4.32	0.17			
Multifocality	2.46	1.22-4.95	0.01	1.90	0.93-3.87	0.08
Polypoid morphology	0.27	0.13-0.57	0.001	0.31	0.14-0.65	0.002
Invisible dysplasia	1.64	0.76-3.53	0.21			
Nonpolypoid morphology	1.82	0.70-4.74	0.22			

*advanced colorectal neoplasia, aCRN: defined as colorectal cancer and/or high-grade dysplasia



Supplementary Figure 1.Kaplan-Meier time-to-event (aCRN*) analysis for patients with indeterminate dysplasia (IND); time from first IND within study interval to event. (p=0.02, log-rank test).

CHAPTER 3

Clinical implications of low grade dysplasia found during inflammatory bowel disease surveillance: a retrospective study comparing chromoendoscopy and white-light endoscopy

Endoscopy, 2017

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ABSTRACT

Background and study aims: Current guidelines recommend the use of pancolonic chromoendoscopy for surveillance of patients with inflammatory bowel disease (IBD). It is currently unknown whether low grade dysplasia (LGD) found using chromoendoscopy carries a similar risk of high grade dysplasia (HGD) or colorectal cancer (CRC) compared with LGD detected using white-light endoscopy (WLE). The aim of this study was to compare the risk of advanced neoplasia, a combined endpoint of HGD and CRC, during follow-up after detection of lesions containing LGD identified with either chromoendoscopy or WLE.

Patients and methods: A retrospective cohort was established to identify patients who underwent IBD surveillance for ulcerative colitis or colonic Crohn's disease between 2000 and 2014. Subgroups were identified, based on the endoscopic technique (standard definition resolution WLE, high definition resolution WLE or chromoendoscopy). LGD detected in random biopsies was considered invisible LGD. Patients were followed until detection of advanced neoplasia, colectomy, death, or the last known surveillance colonoscopy.

Results: Of 1065 patients undergoing IBD surveillance, 159 patients underwent follow-up for LGD, which was visible in 133 cases and invisible in 26 cases. On follow-up, five cases of HGD and five of CRC were detected. The overall incidence rate of advanced neoplasia was 1.34 per 100 patient-years with a median follow-up of 4.7 years and a median time to advanced neoplasia of 3.3 years. There were no significant differences in the incidence of advanced neoplasia between chromoendoscopy-detected and WLE-detected LGD.

Conclusion: Advanced neoplasia was found to develop infrequently after detection of LGD in patients undergoing endoscopic surveillance for IBD. LGD lesions detected with either chromoendoscopy or WLE carry similar risks of advanced neoplasia over time.

INTRODUCTION

Patients with longstanding extensive ulcerative colitis (UC) or Crohn's colitis are at increased risk of developing colorectal cancer (CRC).[1] Additional risk factors for the development of colitis-associated CRC are: a family history of CRC, early age of inflammatory bowel disease (IBD) onset, a concurrent diagnosis of primary sclerosing cholangitis (PSC), presence of post-inflammatory polyps, and ongoing disease activity.[2,3] Recent studies have estimated that the cumulative risk of CRC in IBD patients is approximately 5% after a disease duration of 20 years or more,[4,5] which is lower than previously reported.[6] The occurrence of CRC is thought to be preceded by neoplastic progression via low grade dysplasia (LGD) and high grade dysplasia (HGD),[7,8] opening a window of opportunity for secondary prevention through surveillance colonoscopies.

Currently, high definition resolution (HDR) colonoscopy, if possible combined with pancolonic dye-spraying (chromoendoscopy), is considered the most sensitive method for the detection of dysplasia in patients with colonic IBD.[9] This is based on evidence showing a higher dysplasia yield for chromoendoscopy than for standard definition resolution white-light endoscopy (SDR-WLE).[10–12] It is, as of yet, unknown if lesions detected using HDR-WLE or chromoendoscopy indicate a similar risk of future advanced neoplasia, defined as HGD or CRC, as those detected by SDR-WLE. The aim of this study was therefore to compare the risk of developing HGD or CRC following the detection of lesions containing LGD during colonoscopic IBD surveillance using WLE and chromoendoscopy.

PATIENTS AND METHODS

Patients with IBD were retrospectively identified from three Dutch tertiary referral centers using diagnosis and treatment combinations which resemble the WHO International Classification of Disease coding system.[13] Patients undergoing endoscopic surveillance between January 2000 and June 2014 were selected. Patients were considered eligible for colonoscopic surveillance if they had had a disease duration of at least 8 years and had involvement of at least 30% of the colonic mucosa. A concomitant diagnosis of PSC was considered an immediate indication for surveillance.

Patients' medical records were reviewed to retrieve demographic data, including IBD type, date of IBD diagnosis, maximum (endoscopic) disease extent, family history of CRC, and history of dysplasia before surveillance. In patients with Crohn's disease, the disease was categorized using the Montreal classification.

Endoscopic technique

Colonoscopies were classified as surveillance endoscopies when the endoscopy report explicitly stated this as the indication for the procedure and when a surveillance protocol included either WLE with random biopsies or the use of chromoendoscopy. At the start of the study period, surveillance colonoscopies were performed using WLE and involved targeted biopsies of any abnormality, along with a random biopsy protocol. Following updates in guidelines, all three participating centers gradually adopted chromoendoscopy as their first-choice modality for IBD surveillance.[9] Chromoendoscopy involves pancolonic dye-spraying using either 0.1% methylene blue or 0.3% indigo carmine along with targeted biopsy of abnormal areas.

Only endoscopists with extensive experience in surveillance of IBD patients performed chromoendoscopy. For each surveillance procedure, the type of colonoscope and the use of pancolonic dye-spraying were retrieved from the endoscopy report. The colonoscope types were stratified based on image quality (SDR-WLE or HDR-WLE), as provided by the manufacturer.

The interval between surveillance colonoscopies was determined using the criteria stated in the updated guidelines of the British Society of Gastroenterology.[9]

Detection of neoplasia

For each colonoscopic procedure, pathology reports were reviewed to identify cases with dysplastic lesions. For each lesion, additional data were collected on size, location, endoscopic morphology, histologic classification, p53 status, and endoscopic management. Patients were enrolled into the study following identification of their first LGD lesion during surveillance (hereafter called the index lesion). All LGD lesions identified through targeted biopsies were considered visible lesions.

The endoscopic technique employed to detect the index lesion was used to stratify the patients into HDR-WLE, SDR-WLE, or chromoendoscopy subgroups for follow-up. All chromoendoscopic colonoscopies were performed using HDR equipment. If the index lesion was found in a random biopsy in the absence of a visible dysplastic lesion, the patient was allocated to a separate subgroup (invisible dysplasia). Lesions detected using random biopsies were considered to be non-resected. If an endoscopic procedure yielded multiple spatially distinct dysplastic lesions, this was considered to be multifocal dysplasia.

Incidence of advanced neoplasia during follow-up

All patients in whom an index lesion was identified were followed up until 1 July 2015. Patients were excluded from further analysis if no follow-up colonoscopy had taken place by this time or if the index lesion had been managed by colectomy rather than endoscopic follow-up.

The incidence of advanced neoplasia was defined as the presence of HGD or CRC, found either during colonoscopy or in a surgical colectomy specimen. Persistence of dysplasia was defined as the presence of LGD found during subsequent surveillance colonoscopies.

All colorectal cancers were coded according to the Dukes' classification. Censoring was performed in case of colectomy, death, or the last known surveillance colonoscopy before 1 July 2015.

Statistical analysis

Baseline data are presented for unique patients rather than for procedures. Dichotomous outcomes are presented as the number of events with a corresponding percentage and were compared by chi-squared testing. Continuous data are presented as a mean with standard deviation (SD) or median and range and were compared by the Student's *t* test or Mann–Whitney *U* test according to normality. Advanced neoplasia-free survival was examined using Kaplan–Meier curves and comparisons were made using Cox proportional hazard modeling. The risk of advanced neoplasia is presented as the number of events per 100 patient-years after identification of the index lesion.

Throughout the entire analysis, two-sided *P* values were set at 0.05 for identification of a statistically significant difference. All data analyses were performed using SPSS version 21 (IBM Corp., Armonk, New York, USA).

RESULTS

Patient selection

Of 1065 patients undergoing surveillance, 196 had LGD at least once in the study period. Of these, 37 patients were excluded because their follow-up after the diagnosis of index dysplasia had not yet taken place. The remaining 159 patients were stratified according to the endoscopic technique used to identify the visible index lesion as follows: SDR-WLE, n = 80; HDR-WLE, n = 21; chromoendoscopic colonoscopy, n = 32; and invisible lesions, n = 26 (Figure 1).

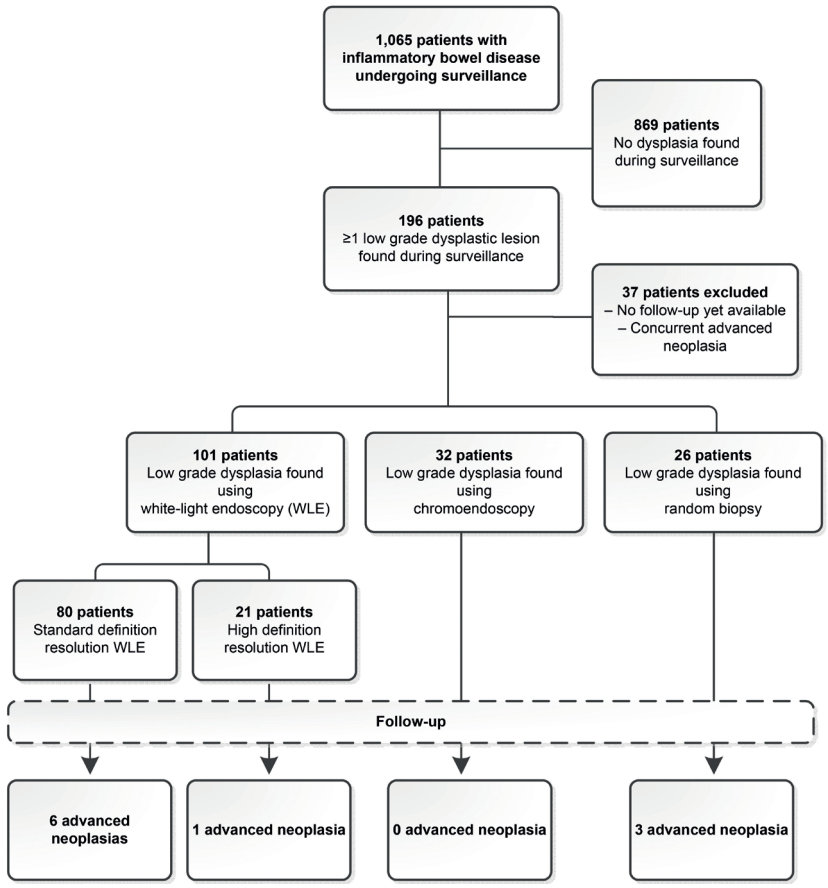


Figure 1. Flowchart of the patients within the study.

Demographics

Baseline demographic and clinical parameters for the patients with visible index lesions are displayed in Table 1. Of all 159 patients, 97 (61.0%) were men and the majority had a diagnosis of UC (57.4%). The mean patient age at the time of detection of their index lesion was 55 years and mean disease duration was 33 years. Most index lesions in the chromoendoscopy group were found after 2010 (81.2%), while index lesions in the other groups were predominantly found before 2010.

Patients were followed for a median of 4.7 years after detection of index dysplasia. The cohort was followed for a total of 749 patient-years after index dysplasia. The

duration of follow-up was significantly shorter for patients in the chromoendoscopy group (2.0 years) compared with the other groups ($P < 0.001$). In the majority of cases (74%), the visible lesions were directly removed endoscopically. Visible lesions that were not resected (26%) consisted of lesions that were deemed endoscopically unresectable (e.g. strictures), as well as lesions that should have been removed completely (e.g. polyps that were initially only biopsied).

Table 1. Baseline characteristics of the 133 patients with inflammatory bowel disease (IBD) who had a visible index lesion containing low grade dysplasia detected, grouped according to surveillance technique used.

	Chromoendoscopy	White-light endoscopy	*P* value
Number of patients	32	101	–
Male sex, n (%)	16 (50%)	65 (64.4%)	0.10
IBD diagnosis			0.75
Ulcerative colitis	19 (59.4%)	54 (53.5%)	
Crohn's colitis	12 (37.5%)	41 (40.6%)	
Indeterminate colitis	1 (3.1%)	6 (5.9%)	
Age, mean ± SD, years	55 ± 11	56 ± 11	0.55
Age at IBD diagnosis, mean ± SD, years	30 ± 11	34 ± 13	0.21
First degree relative with colorectal cancer	3 (10.7%)	7 (6.9%)	0.34
Post-inflammatory polyps	9 (33.3%)	20 (19.8%)	0.24
Primary sclerosing cholangitis	1 (3.6%)	6 (5.9%)	0.53
Index lesion before 2010 (%)	6 (18.8%)	94 (93.1%)	<0.001
Location of index dysplasia (unifocal only)			0.29
Left colon	12 (46.2%)	30 (39.5%)	
Transverse colon	4 (15.4%)	14 (18.4%)	
Right colon	10 (38.5%)	23 (30.3%)	
Data missing	0 (0%)	9 (11.8%)	
Multifocality	6 (18.8%)	24 (23.8%)	0.66
History of dysplasia	4 (14.3%)	10 (9.9%)	0.36
Repeated finding of dysplasia	13 (40.6%)	43 (42.6%)	0.51

SD, standard deviation.

Incidence of advanced neoplasia during follow-up

The median time to the occurrence of advanced neoplasia was 3.3 years for the whole cohort. Following detection of the index lesion, 10 patients developed an advanced neoplastic lesion (HGD, n = 5; CRC, n = 5), with an incidence rate of 1.34 cases per 100 patient-years (Table 2). Kaplan–Meier curves for advanced neoplasia-free survival for all patients and for the separate groups are displayed in Figure 2.

Table 2. Incidence of advanced neoplasia after detection of low grade dysplasia (LGD).

	Overall	Chromoendoscopy	HDR-WLE	SDR-WLE	Invisible
Number of patients	159	32	21	80	26
Follow-up, median (range), years	4.7 (0.2–12.6)	2.0 (0.9–5.3)	4.2 (0.2–7.8)	5.9 (0.2–11.5)	4.7 (0.5–12.6)
Advanced neoplasia, n (%) (CRC/HGD)	10 (6.3%) (5/5)	0 (0.0%)	1 (4.8%) (1/0)	6 (7.5%) (3/3)	3 (11.5%) (1/2)
Advanced neoplasia incidence rate, cases per 100 patient-years	1.34	0.0 0.97 (all visible lesions)	1.24	1.29	2.29
Time to advanced neoplasia, median, years	3.2	–	1.1	3.2	3.8

HDR-WLE, high definition resolution white-light endoscopy; SDR-WLE, standard definition resolution white-light endoscopy; CRC, colorectal cancer; HGD, high grade dysplasia.

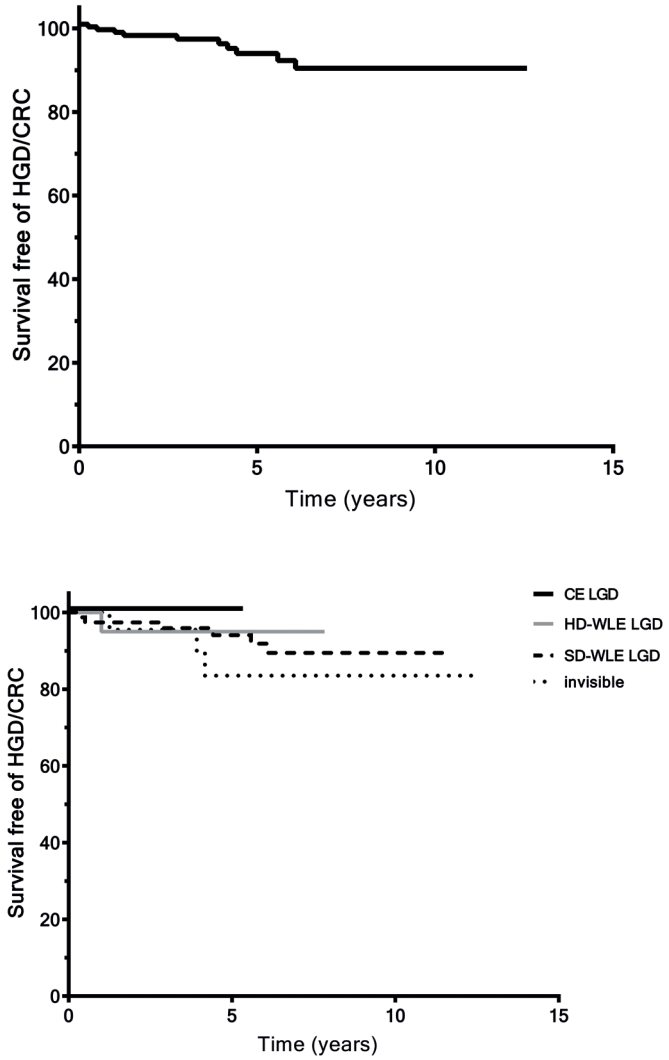


Figure 2. Advanced neoplasia-free survival after detection of low grade dysplasia for: **a** all 159 patients; **b** patients with lesions detected by chromoendoscopy (n = 32), high definition resolution white-light endoscopy (WLE; n = 21), standard definition resolution WLE (n = 80), and on random biopsy only (invisible lesions; n = 26). (HGD, high grade dysplasia; CRC, colorectal cancer.)

There were no significant differences in the incidence rates of advanced neoplasia between the groups based on endoscopic method of identification (log rank test, *P* = 0.73). The advanced lesion emerged in the same colonic segment as the index

lesion in 7 of 10 patients. Five of seven visible lesions with advanced neoplasia on follow-up (71%) were reported to have been removed endoscopically, while two lesions were incompletely removed. When the visible index lesions only were considered, the incidence rate was 0.97 per 100 patient-years. In the chromoendoscopy group, no advanced neoplasia was observed over a median period of 24 months. In the HDR-WLE group, one patient developed CRC in the same colonic segment 13 months after the index dysplasia. In this patient, the index lesion was incompletely removed and this was later considered to have progressed to the advanced lesion. Timelines for patients with advanced neoplasia after index dysplasia are displayed in Figure 4.

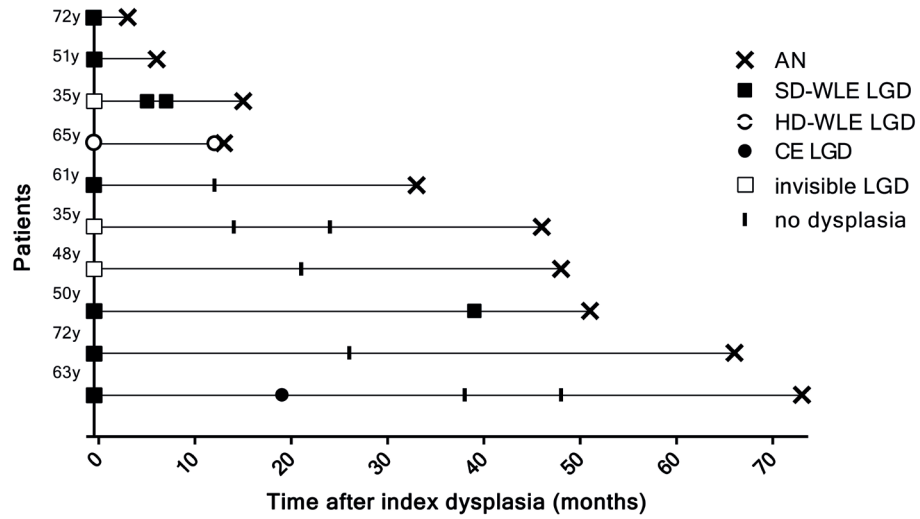


Figure 4. Timelines for patients with advanced neoplasia after index dysplasia. All invisible LGD was detected in random biopsies taken during SD-WLE procedures.

Invisible dysplasia

In 26 patients, the index lesion was LGD in a random biopsy, without a synchronous visible dysplastic lesion. The incidence rate for advanced neoplasia in this subgroup was 2.29 per 100 patient-years (*P* = 0.276 compared with visible lesions). In five patients, LGD was detected in one or more biopsies taken from mucosa surrounding a visible dysplastic lesion. In none of these patients was advanced neoplasia found during follow-up.

A breakdown of all the different endoscopic surveillance procedures with dysplasia, including those performed after index dysplasia, is provided in Table 3.

Table 3. Comparison of endoscopic procedures in which one or more foci of visible low grade dysplasia were seen.

	Chromoendoscopy	HDR-WLE	SDR-WLE	*P* value
Total number of procedures	95	57	115	–
Number of visible dysplastic lesions per procedure	1.5	1.7	1.3	0.13
Number of dysplastic foci				0.1
Unifocal	68 (71.6%)	33 (57.9%)	84 (73.0%)	
Multifocal	27 (28.4%)	24 (42.1%)	31 (27.0%)	

HDR-WLE, high definition resolution white-light endoscopy; SDR-WLE, standard definition resolution white-light endoscopy.

The results of univariate analysis to identify additional risk factors for the occurrence of advanced neoplasia during follow-up are displayed in Table 4. None of the examined variables were found to have a significant association with the occurrence of advanced neoplasia.

Table 4. Univariate analysis of factors potentially associated with the incidence of advanced neoplasia on follow-up of patients with inflammatory bowel disease (IBD).

Variable	Hazard ratio (95% confidence interval)	*P* value
Male sex	2.35 (0.50–11.12)	0.28
IBD type, ulcerative colitis	1.13 (0.32–4.02)	0.85
Age at index lesion, years	1.00 (0.99–1.01)	0.88
Age at index lesion >50 years	1.29 (0.33–5.01)	0.71
Age at IBD diagnosis	1.00 (0.99–1.01)	0.85
Age at IBD diagnosis <30 years	0.49 (0.14–1.74)	0.27
Duration of IBD	1.00 (0.94–1.07)	0.92
Duration of IBD >15 years	1.00 (0.26–3.88)	>0.99
Post-inflammatory polyps	0.60 (0.11–3.23)	0.55
Primary sclerosing cholangitis	1.82 (0.23–14.42)	0.57
Visible lesion	0.48 (0.12–1.86)	0.29
Distal location of index dysplasia	1.51 (0.30–7.48)	0.62
Multifocal lesions	2.18 (0.56–8.45)	0.28
Repeated finding of dysplasia	0.30 (0.06–1.40)	0.12

Endoscopically removed index lesions

All visible index lesions that were reported to have been removed endoscopically were analyzed separately. After excluding invisible lesions and lesions that were initially only biopsied, 89 visible index lesions remained (SDR-WLE, n = 52; HDR-WLE, n = 17; chromoendoscopic colonoscopy, n = 20). Within this group, five patients developed advanced neoplasia, all of whom had an index lesion detected by SDR-WLE. No advanced neoplasia was observed after endoscopic removal of the index lesion in either the HDR-WLE or chromoendoscopy group (Figure 3).

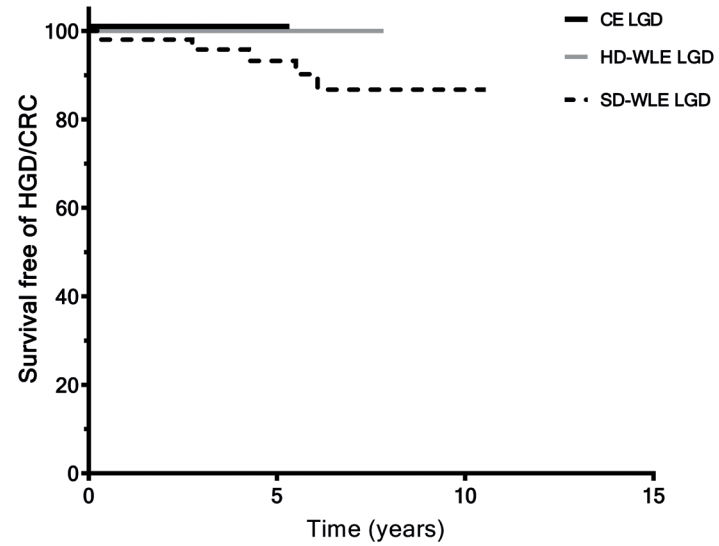


Figure 3. Advanced neoplasia-free survival for visible lesions that were endoscopically removed (n = 89). (HGD, high grade dysplasia; CRC, colorectal cancer.)

Table 5. Overview of recent studies on the development of advanced neoplasia after detection of low grade dysplasia.

Author	Year	Study period	Patients with low grade dysplasia (visible/ invisible)	Advanced neoplasia (high grade dysplasia or colorectal cancer)	Incidence rate (per 100 patient-years)
Thomas et al. (meta-analysis) [16]	2007	1982–2003	508 (31/477)	65 (12.8%)	3.0
Zisman et al. [15]	2012	1987–2002	42 (23/19)	8 (19.0%)	Not stated
Navaneethan et al. [17]	2013	1998–2011	102 (65/37)	5 (4.9%)	2.1 (distal) 0.5 (proximal)
Venkatesh et al. [14]	2013	1996–2011	10 (only PSC) (3/7)	3 (30%)	9.4
Choi et al. [18]	2015	1993–2012	172 (155/16)	33 (19.2%)	3.9
Wanders et al. (postpolypectomy meta-analysis) [19]	2014	1975–2008	376 (376/0)	12 (3.2%)	0.7

PSC, primary sclerosing cholangitis.

DISCUSSION

The diagnosis of LGD in the setting of IBD surveillance has been associated with a substantial risk of progression to advanced neoplasia.[14,15] However, we found a modest overall incidence rate of 1.34 per 100 patient-years for all LGD lesions, 0.97 per 100 patient-years for visible lesions and 2.29 per 100 patient-years for invisible lesions. Furthermore, the incidence of advanced neoplasia for patients with LGD index lesions detected with either chromoendoscopy or HDR-WLE was not different.

The majority of reports on the natural history of LGD in IBD patients originate from an era in which most dysplasia was considered macroscopically invisible and endoscopically unresectable. Consequently, the occurrence of CRC during follow-

up was considered to be neoplastic progression of these lesions. In a meta-analysis by Thomas et al. published in 2007, pooled results of 20 studies (1982–2003) showed progression rates of 1.4 per 100 patient-years for CRC and 3.0 per 100 patient-years for a combined endpoint of dysplasia-associated lesion or mass (DALM), HGD, and CRC.[16] Studies on the natural history of LGD published since this review have reported progression rates ranging from 4.9% to 30% (Table 5). Several factors have been found to increase the risk of progression, including the presence of multifocal lesions,[15] distal localization,[17,18] and a concurrent diagnosis of PSC.[14] Our study found lower incidence rates than previously reported and we were not able to reproduce these characteristics as independent risk factors for advanced neoplasia.

Recently, Wanders et al. performed a meta-analysis on the risk of CRC after complete resection of polypoid dysplasia, incorporating 10 studies, and calculated a pooled incidence ratio of 0.7 for HGD and CRC combined.[19] This number is far lower than reported in studies that included invisible dysplasia and is more in line with our findings. The higher incidence rate for visible lesions in our study (0.97) may be due to the fact that we included all lesions containing LGD, whereas Wanders et al. selectively looked at conventional polypoid dysplastic lesions.

In our cohort, lesions that were reported as "indefinite for dysplasia" were not selected as index lesions. However, a recent study by Lai et al. reported an incidence of 1.5 cases of advanced neoplasia per 100 patient-years for patients with indefinite-for-dysplasia lesions, which is similar to the incidence rate for LGD in this study.[20] Van Schaik et al. found a 5-year progression rate of 19% for invisible LGD and 21% for indefinite-for-dysplasia lesions, which was corrected to 37% and 5%, respectively, after review of the histologic slides.[21] Although details on the types of colonoscope used were not available in this study, or in other reports on the follow-up of LGD, it can be assumed that SDR colonoscopes were used in this study.

Of primary interest in the current study were the patients with index lesions found using newer endoscopic techniques such as HDR-WLE and chromoendoscopy. Chromoendoscopy has repeatedly been shown to have an increased dysplasia detection rate when compared with WLE in a study setting. It should be noted

however that in most trials in which chromoendoscopy was studied, comparison was made with a group of patients undergoing SDR-WLE. Whether HDR-WLE without scheduled random biopsies will yield the same results as chromoendoscopy remains to be proven. Moreover, the clinical significance of lesions found with chromoendoscopy has not been established.[22] Some authors have hypothesized that these smaller lesions may be less advanced and therefore may have less malignant potential, yet no strong evidence in support of this hypothesis has been published to date.

In this study, we did not find a significant difference in the risk of advanced neoplasia during follow-up for index lesions detected with either WLE or chromoendoscopy, nor did we find additional risk factors regarding the index colonoscopy that were associated with the occurrence of advanced neoplasia during follow-up. On the basis of these results, the risk is similar for each LGD lesion irrespective of the endoscopic method used to detect it, although the low number of advanced neoplastic lesions may have caused a lack of power to detect more subtle differences.

A clear distinction was made between visible lesions and invisible lesions found in random biopsies, historically referred to as flat dysplasia (fLGD). Invisible dysplasia managed by endoscopic follow-up was an important subgroup in our study, as the incidence rate of advanced neoplasia was highest in these patients. This higher incidence rate may be explained by the fact that residual dysplastic mucosa was undoubtedly present in these patients. Nonetheless, it cannot be excluded that this was the result of a field cancerization effect.[23] Interestingly, however, in patients in whom biopsies from surrounding mucosa were positive for dysplasia, no advanced neoplasia occurred during follow-up.

In 23 of 26 patients, the invisible index lesions were detected before 2010. It can be argued that these lesions, previously considered invisible, would have been visible lesions in the current era following improvements in image quality, which may have rendered them amenable to resection.

This trend has already been seen in clinical practice, as there has been a shift in the management of LGD from a surgical approach towards endoscopic removal.[24,25] We excluded patients who underwent direct colectomy for LGD and it is possible that including these patients would have increased the overall risk of advanced neoplasia during follow-up in our cohort.

We found a significantly lower incidence rate of advanced neoplasia following the identification of LGD lesions as compared to previous studies.[1,26] The observed general risk of CRC in IBD patients in the current era is lower than previously reported[5], which may reflect improved control of inflammation through the increasing use of immunomodulators or biologicals. Second, the risk of advanced neoplasia may have been reduced owing to the fact that surveillance has reached a new level of effectiveness in detecting and removing precancerous lesions. Timely planning of follow-up procedures coupled with complete endoscopic removal of lesions will reduce most future development of CRC.[27]

The strengths of the present study include the relatively large cohort, verification of the colonoscope type used for each procedure, inclusion of patients with both UC and Crohn's colitis, and distinction between visible and invisible dysplasia. Moreover, the results are based on high quality procedures that were performed by experienced endoscopists with a special interest in IBD.

There are also some limitations to this study. First, the number of events per subgroup was relatively small, despite the large number of patients and colonoscopies included. Second, the follow-up time for the HDR-WLE and chromoendoscopy groups was limited owing to the fact that both techniques were only recently introduced. Patients in the SDR-WLE group had a longer follow-up period, with an average of 6 years. However, there are no clear indications from studies with longer follow-up times that the relative risk of advanced neoplasia is influenced by the length of the follow-up period.[19,28] Third, because of the retrospective design of this study, it was not always possible to discern whether biopsies containing dysplastic lesions originated from previously inflamed mucosa. A proportion of the included lesions may have consisted of sporadic adenomas, which are considered to have a lower risk of

neoplastic progression than colitis-associated dysplasia.[3] Fourth, it was in most cases not possible to directly link index lesions with advanced neoplasia found later on, especially because the majority of lesions were removed upon first detection. Apart from the index procedure, findings during subsequent surveillance colonoscopies are expected to further aid in determining the risk of advanced neoplasia for individual patients.[29]

In summary, we observed a low rate of advanced neoplasia on follow-up after detection of LGD during IBD surveillance. This study shows no clear difference in outcomes for LGD detected by chromoendoscopy or HDR-WLE. A prospective study comparing these techniques head-to-head is needed to confirm whether the clinical significance of these lesions is indeed comparable. Our results support the notion that colectomy is no longer indicated for lesions that can be endoscopically resected.

REFERENCES

1. Bernstein, C. N., Blanchard, J. F., Kliewer, E. & Wajda, A. Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* **91,** 854–62 (2001).
2. Soetikno, R. M., Lin, O. S., Heidenreich, P. A., Young, H. S. & Blackstone, M. O. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. *Gastrointest. Endosc.* **56,** 48–54 (2002).
3. Choi, C.-H. R. *et al.* Forty-Year Analysis of Colonoscopic Surveillance Program for Neoplasia in Ulcerative Colitis: An Updated Overview. *Am. J. Gastroenterol.* **110,** 1022–34 (2015).
4. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
5. Jess, T. *et al.* Decreasing risk of colorectal cancer in patients with inflammatory bowel disease over 30 years. *Gastroenterology* **143,** 375-81–4 (2012).
6. Eaden, J. A., Abrams, K. R. & Mayberry, J. F. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* **48,** 526–35 (2001).
7. Feagins, L. A., Souza, R. F. & Spechler, S. J. Carcinogenesis in IBD: potential targets for the prevention of colorectal cancer. *Nat. Rev. Gastroenterol. Hepatol.* **6,** 297–305 (2009).
8. Riddell, R. H. *et al.* Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. *Hum. Pathol.* **14,** 931–68 (1983).
9. Cairns, S. R. *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* **59,** 666–689 (2010).
10. Marion, J. F. *et al.* Chromoendoscopy is More Effective Than Standard Colonoscopy in Detecting Dysplasia During Long-term Surveillance of Patients with Colitis. *Clin. Gastroenterol. Hepatol.* (2015). doi:10.1016/j.cgh.2015.11.011
11. Subramanian, V., Mannath, J., Ragunath, K. & Hawkey, C. J. Meta-analysis: the diagnostic yield of chromoendoscopy for detecting dysplasia in patients with colonic inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **33,** 304–12 (2011).
12. Kiesslich, R. *et al.* Methylene blue-aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology* **124,** 880–8 (2003).
13. Mooiweer, E. *et al.* Chromoendoscopy for Surveillance in Inflammatory Bowel Disease Does Not Increase Neoplasia Detection Compared With Conventional Colonoscopy With Random Biopsies: Results From a Large Retrospective Study. *Am. J. Gastroenterol.* **110,** 1014–21 (2015).
14. Venkatesh, P. G. K., Jegadeesan, R., Gutierrez, N. G., Sanaka, M. R. & Navaneethan, U. Natural history of low grade dysplasia in patients with primary sclerosing cholangitis and ulcerative colitis. *J. Crohn's Colitis* **7,** 968–973 (2013).

15. Zisman, T. L. *et al.* Prospective study of the progression of low-grade dysplasia in ulcerative colitis using current cancer surveillance guidelines. *Inflamm. Bowel Dis.* **18,** 2240–2246 (2012).

16. Thomas, T., Abrams, K. A., Robinson, R. J. & Mayberry, J. F. Meta-analysis: cancer risk of low-grade dysplasia in chronic ulcerative colitis. *Aliment. Pharmacol. Ther.* **25,** 657–68 (2007).

17. Navaneethan, U. *et al.* Progression of low-grade dysplasia to advanced neoplasia based on the location and morphology of dysplasia in ulcerative colitis patients with extensive colitis under colonoscopic surveillance. *J. Crohns. Colitis* **7,** e684-91 (2013).

18. Goldstone, R., Itzkowitz, S., Harpaz, N. & Ullman, T. Progression of low-grade dysplasia in ulcerative colitis: effect of colonic location. *Gastrointest. Endosc.* **74,** 1087–93 (2011).

19. Wanders, L. K. *et al.* Cancer risk after resection of polypoid dysplasia in patients with longstanding ulcerative colitis: a meta-analysis. *Clin. Gastroenterol. Hepatol.* **12,** 756–64 (2014).

20. Lai, K. K. *et al.* Risk for Colorectal Neoplasia in Patients with Inflammatory Bowel Disease and Mucosa Indefinite for Dysplasia. *Inflamm. Bowel Dis.* **21,** 378–384 (2015).

21. van Schaik, F. D. M. *et al.* Misclassification of dysplasia in patients with inflammatory bowel disease: consequences for progression rates to advanced neoplasia. *Inflamm. Bowel Dis.* **17,** 1108–16 (2011).

22. Marion, J. F. & Sands, B. E. The SCENIC Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease: Praise and Words of Caution. *Gastroenterology* **148,** 462–467 (2015).

23. Leedham, S. J. *et al.* Clonality, founder mutations, and field cancerization in human ulcerative colitis-associated neoplasia. *Gastroenterology* **136,** 542–50.e6 (2009).

24. Lim, C. H. *et al.* Ten year follow up of ulcerative colitis patients with and without low grade dysplasia. *Gut* **52,** 1127–32 (2003).

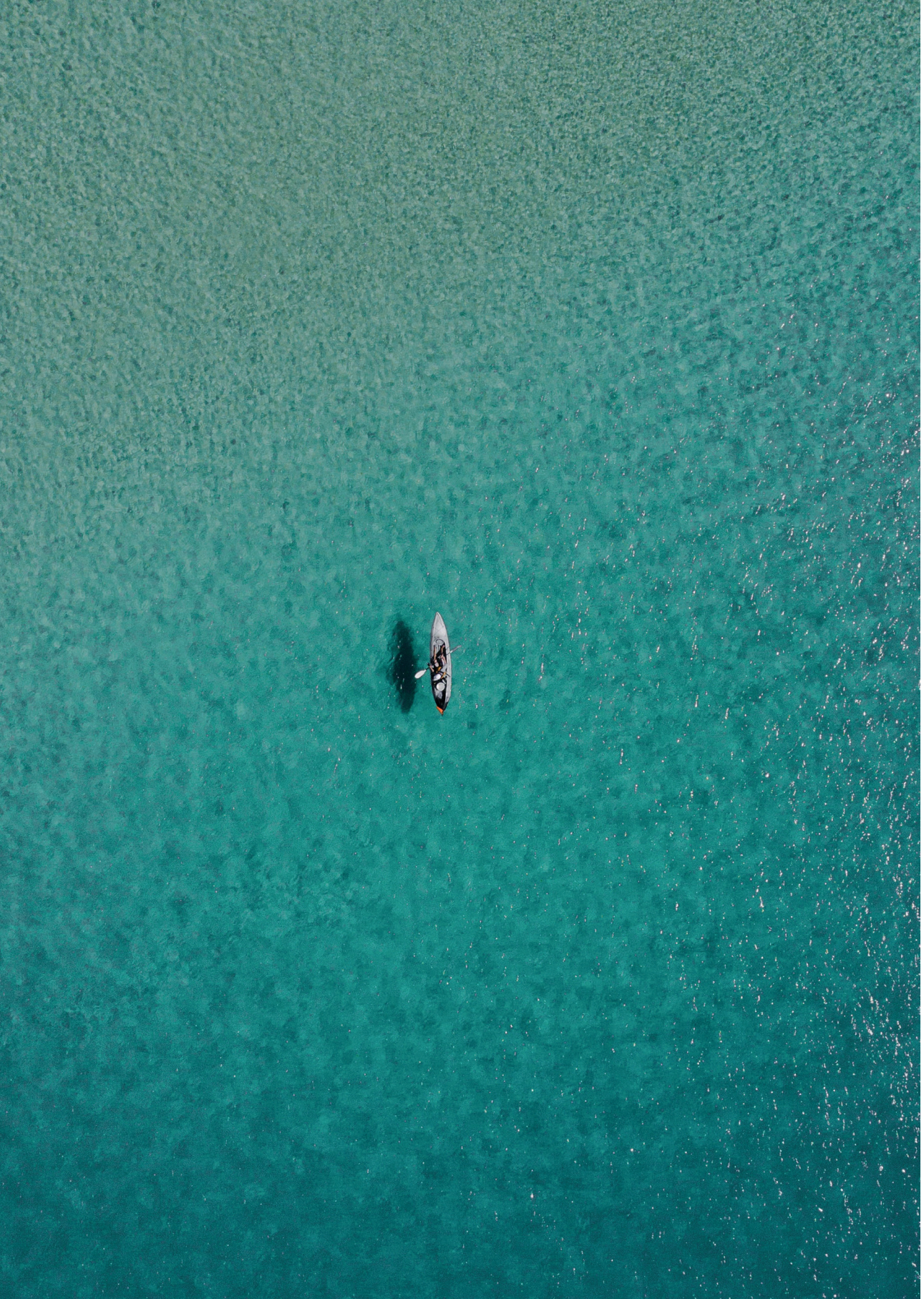
25. Befrits, R., Ljung, T., Jaramillo, E. & Rubio, C. Low-grade dysplasia in extensive, long-standing inflammatory bowel disease: a follow-up study. *Dis. Colon Rectum* **45,** 615–20 (2002).

26. Blackstone, M. O., Riddell, R. H., Rogers, B. H. & Levin, B. Dysplasia-associated lesion or mass (DALM) detected by colonoscopy in long-standing ulcerative colitis: an indication for colectomy. *Gastroenterology* **80,** 366–74 (1981).

27. Mooiweer, E. *et al.* Incidence of Interval Colorectal Cancer Among Inflammatory Bowel Disease Patients Undergoing Regular Colonoscopic Surveillance. *Clin. Gastroenterol. Hepatol.* **13,** 1656–1661 (2015).

28. Choi, C.-H. R. *et al.* Low-Grade Dysplasia in Ulcerative Colitis: Risk Factors for Developing High-Grade Dysplasia or Colorectal Cancer. *Am. J. Gastroenterol.* **110,** 1461–71 (2015).

29. Rutter, M. D. *et al.* Cancer surveillance in longstanding ulcerative colitis: endoscopic appearances help predict cancer risk. *Gut* **53,** 1813–1816 (2004).



CHAPTER 4

Consecutive negative findings on colonoscopy during surveillance predict a low risk of advanced neoplasia in inflammatory bowel disease patients with longstanding colitis: results of a 15-year multicenter, multinational cohort study

Gut, 2019

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ABSTRACT

Objectives: Surveillance colonoscopy is thought to prevent colorectal cancer (CRC) in patients with longstanding colonic inflammatory bowel disease (IBD), but data regarding the frequency of surveillance and the findings thereof are lacking. Our aim was to determine whether consecutive negative surveillance colonoscopies adequately predict low neoplastic risk.

Design: A multicenter, multinational database of long-standing IBD colitis patients without high-risk features and undergoing regular CRC surveillance was constructed. A "negative" surveillance colonoscopy was predefined as a technically adequate procedure having no post-inflammatory polyps, no strictures, no endoscopic disease activity and no evidence of neoplasia; a "positive" colonoscopy was a technically adequate procedure that included at least one of these criteria. The primary endpoint was advanced colorectal neoplasia (aCRN), defined as high-grade dysplasia or CRC.

Results: Of 775 patients with long-standing IBD colitis, 44% (n=340) had ≥1 negative colonoscopy. Patients with consecutive negative surveillance colonoscopies were compared to those who had at least one positive colonoscopy. Both groups had similar demographics, disease-related characteristics, number of surveillance colonoscopies and time intervals between colonoscopies. No aCRN occurred in those with consecutive negative surveillance, compared to an incidence rate of 0.29 to 0.76/100 patient-years (p=0.02) in those having ≥1 positive colonoscopy on follow-up of 6.2 (P25-P75: 4.4-8.2) years after the index procedure.

Conclusion: Within this large surveillance cohort of patients with colonic IBD and no additional high-risk features, having two consecutive negative colonoscopies predicted a very low risk of aCRN occurrence on follow-up. Our findings suggest that longer surveillance intervals in this selected population may be safe.

Significance of this study

What is already known about this subject?

- Because long-standing colonic inflammatory bowel disease (IBD) is associated with an increased risk of colorectal cancer (CRC), the current clinical recommendation is for interval colonoscopy for CRC surveillance.
- The recommended interval for CRC surveillance in this population ranges anywhere from 1-5 years, and most often every 1-2 years, depending on clinical and endoscopic factors.
- Colonoscopic surveillance, which is associated with increased cost, patient inconvenience, and small procedural risk, is of uncertain benefit in patients with no additional risk factors for CRC and well-controlled IBD with quiescent endoscopic disease on follow-up.

What are the new findings?

- Using a large tri-national and multicenter database of patients with confirmed colonic IBD undergoing colonoscopic surveillance for CRC, we demonstrated that patients without high-risk demographics (e.g. primary sclerosing cholangitis (PSC), personal or family history of colorectal neoplasia), undergoing adequate quality-metric surveillance colonoscopy without high-risk findings on exam, such as active inflammation, stricture, post-inflammatory polyps, and dysplasia (i.e. a "negative" colonoscopy) had a very low risk of future high-grade dysplasia or CRC.
- Of the cohort without high-risk features like PSC, no one with two consecutive negative surveillance colonoscopies developed high-grade dysplasia or CRC on follow-up.

How might it impact on clinical practice in the foreseeable future?

- Our findings inform current surveillance guidelines for the IBD population and suggest that in patients without additional risk factors for CRC and with at least two consecutive surveillance exams showing endoscopically quiescent disease and no high-risk features, an interval longer than 2 years between surveillance examinations, may be appropriate.
- A longer interval would not only optimize the cost and resource-to-benefit ratio of surveillance, but would improve the quality of life for a large percentage of IBD patients enrolled in surveillance.

INTRODUCTION

Patients with long-standing colonic inflammatory bowel disease (IBD) are at an increased risk of developing colorectal cancer (CRC).[1,2] Colonoscopic surveillance is widely used in these patients to prevent CRC-related mortality. Current guidelines use individual risk stratification to determine patient-specific surveillance intervals, ranging from 1 to 5 years.[3–5] The European and British guidelines have incorporated a low-risk category for patients who have no high- or intermediate-risk features—such as primary sclerosing cholangitis (PSC), active inflammation, or prior history of neoplasia—after an initial screening colonoscopy and allow for a 5-year surveillance interval in this selected population. This is in contrast to practice in North America, where there are no distinct recommendations for the low-risk group, who are instead subjected to an every 1 to 2-year colonoscopic surveillance protocol. This contributes a burden to health care systems in terms of costs and capacity, and to patients in terms of procedural risks, inconvenience and personal costs.

Several large well-designed studies in patients without IBD provide evidence-based guidance on the risk of CRC after negative investigations and inform clinical practice.[6–10] For example, a recent study that included over 2100 average-risk patients found that a adequate quality-metric screening colonoscopy with negative results had significantly lower rates of adenomas or advanced neoplasms and no cancers at their 10-year follow-up colonoscopy, compared to people undergoing their first screening colonoscopy, with the authors concluding that a 10-year interval for CRC screening after a negative colonoscopy is safe in the average-risk population.[10] Furthermore, British screening colonoscopy guidelines suggest that after removal of sporadic adenomas, having two subsequent negative surveillance colonoscopies suggests that future exams can be discontinued altogether.[11] So far, data justifying longer surveillance intervals for low-risk IBD patients, however, are lacking. We sought to address this question by using a multinational, multicenter database of patients with long-standing IBD colitis and no other high-risk features undergoing colonoscopic CRC surveillance. The overall aim was to determine whether two consecutive surveillance colonoscopies that demonstrated no intermediate- to high-risk findings

(according to strict criteria) might predict absent or low rates of advanced colorectal neoplasia (aCRN), defined as high-grade dysplasia (HGD) or CRC, during follow-up.

METHODS

Study population and case identification

Patients undergoing colonoscopic surveillance for IBD colitis between 2000-2015 were retrospectively identified from three databases: a Dutch (Netherlands, NL) database that included 2 secondary and 6 tertiary centers; a United States (US) database that included 1 tertiary center; and a Canadian database including 1 tertiary center. Cases were identified through a query of the electronic health record (EHR)-linked database for all cases of IBD, or through the individual center's patient registry.

Patient selection (inclusion and exclusion criteria)

After initial identification through the EHR query, individual charts were reviewed. Inclusion criteria were: 1) diagnosis of IBD (ulcerative colitis (UC), Crohn's disease (CD), IBD undifferentiated (IBD-U)) with colonic involvement confirmed by pathology; 2) confirmed colonic disease duration of at least 8 years (referred to hereafter as "long-standing"); 3) enrollment in a dysplasia surveillance program; 4) at least two separate colonic pathology reports from surveillance exams; 5) at least left-sided disease extent (UC or IBD-U) or involvement of >30% of the colonic surface (CD or IBD-U); and 6) no aCRN prior to, or at, index colonoscopy during the defined study period. Exclusion criteria were: 1) CD without colonic involvement; 2) UC or IBD-U with limited proctitis and CD or IBD-U with <30% colonic involvement; and 3) less than two surveillance colonoscopies with available pathology. So-called "high risk" patients were also excluded if they had any of the following *a priori* determined factors: PSC, history of any dysplasia or CRC prior to, or at, index colonoscopy, prior history of stricture, or first-degree relative with a history of CRC.

Date of enrollment was the date of the first surveillance colonoscopy within the stated study period (hereafter referred to as the "index colonoscopy"). Surveillance procedures were defined as colonoscopies in which either segmental random

biopsies or chromoendoscopy were employed. We excluded subjects in whom the index or subsequent colonoscopy had inadequate bowel preparation according to the endoscopists' overall impression and/or cecal intubation was not achieved, as these procedures are considered technically insufficient for surveillance. We also excluded any colonoscopies that were performed for an indication other than surveillance, e.g. medically refractory disease, or those that were performed with surveillance as the indication but did not employ at least segmental biopsies or chromoendoscopy. Thus, we only included patients who had at least two consecutive *surveillance* colonoscopies with adequate quality metrics, followed by at least one mode of pathologic assessment on subsequent follow-up—either another surveillance colonoscopy, a colonoscopy for medical refractory disease where biopsies were taken, or colectomy (segmental, subtotal, total)—to determine whether or not there was an eventual diagnosis of dysplasia and/or CRC.

Any interval aCRN diagnosis, irrespective of diagnostic setting (e.g. colectomy), was recorded.

Data collection

The following baseline demographic and clinical data were abstracted: date of birth, sex, age of IBD diagnosis, IBD type (UC, CD, or IBD-U), maximum extent of colonic disease at any time during follow-up, family history of CRC, diagnosis of PSC by endoscopic and/or radiographic cholangiography (ERCP and/or MRCP). Medication exposure was defined as duration of use for at least three months according to EHR documentation and was recorded for 5-aminosalicylates (5-ASA), immunomodulators (azathioprine or 6-mercaptopurine), and biologics.

Details of the index surveillance colonoscopy and each subsequent surveillance exam were recorded, including quality of bowel preparation as determined by the endoscopist at the time of the exam (adequate [excellent or good] or inadequate [fair or poor], the latter of which were excluded), use of chromoendoscopy, most proximal colonic extent examined (with exclusion of those procedures where cecal intubation was not achieved), overall impression of endoscopic inflammation (none

or remission; mildly active; moderately active; severely active), presence of post-inflammatory polyps ("pseudopolyps"), stricture(s) and visible lesions. Lesion location was categorized as left-sided if located distal to the splenic flexure and right-sided if located proximal to the splenic flexure. The number of surveillance colonoscopies, the time interval between exams, and the duration of active follow-up with the treating gastroenterologist were recorded. A standard data collection form was used at all centers.

Histologic data

All histologic diagnoses were recorded as detailed in the original pathology report; no specimens were re-reviewed or altered for the purpose of this study. Dysplasia was reported as indefinite (IND), low-grade (LGD), or high-grade (HGD). For this study, any lesion graded as LGD or higher was defined as neoplasia; aCRN included any HGD or CRC. IND was categorized as non-neoplastic. Of note, it is routine practice at all institutions participating in this study that a specimen concerning for neoplasia (including indeterminate dysplasia) be reviewed by two pathologists and consensus reached before final reporting in the EHR.

Stratification of index colonoscopies

Patients who met inclusion criteria were first assigned to one of two groups based on their initial surveillance colonoscopy within the studied timeframe—either the negative (NEG) index colonoscopy group or the positive (POS) index colonoscopy group—and then subsequently categorized according to findings on their second surveillance colonoscopy, as either NEG-NEG ("double negative") if the two consecutive colonoscopies were negative (as defined below) or NEG-POS, POS-NEG, or POS-POS if the index and/or consecutive colonoscopies included any of the positive findings as detailed.

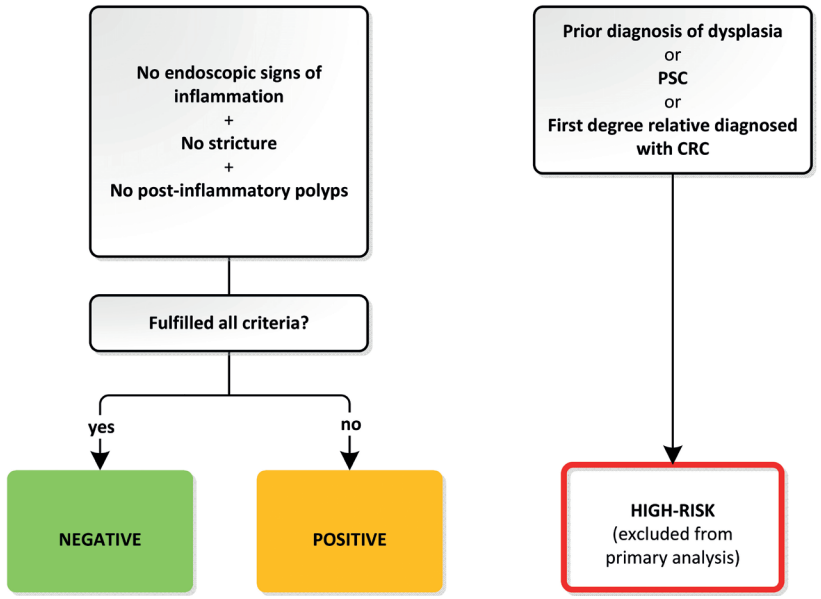


Figure 1. Group allocation algorithm (index procedure). All colonoscopic procedures (positive or negative) had cecal intubation and adequate bowel prep.

For this study, a "negative" colonoscopy was strictly defined as a completed surveillance colonoscopy with no post-inflammatory polyps, no strictures, absence of any endoscopic disease activity, and no dysplasia (Figure 1). If any of these criteria was not met, the colonoscopy was considered "positive". If neoplasia (LGD or higher) was identified on the surveillance procedure following the index procedure ("second colonoscopy", Figure 3), this was recorded, but these patients were excluded from the primary analysis assessing the subsequent development of neoplasia following two surveillance procedures. These patients were instead allocated to the high-risk group and analyzed separately (see below). As noted, patients deemed to be at high-risk for dysplasia and CRC at the outset (e.g. concomitant PSC, prior history of dysplasia, family history of CRC) were excluded from the primary analysis, but were analyzed in a separate analysis (see below).

Primary and secondary outcomes

The primary outcome was occurrence of aCRN following consecutive surveillance colonoscopies after the index examination. Secondary outcomes included any neoplastic diagnosis during follow-up (i.e. LGD or higher), and a diagnosis of aCRN following *any* two negative surveillance exams during the follow-up period. Patients were censored at the time of aCRN diagnosis, colectomy, the date of the last gastroenterology follow-up, or the end of the predetermined study interval, whichever occurred first.

Statistical analysis

Descriptive statistics were generated for patients meeting the inclusion criteria. Chi-square test was used for categorical variables and the Student's t-test for continuous variables. The primary and secondary outcomes were analyzed using a time-to-event analysis and Kaplan-Meier survival curves were generated to compare the various groups according to their surveillance colonoscopy findings. The incidence rate of neoplasia and aCRN was reported as the number of cases per 100 patient-years of follow-up (pty) with 95% confidence interval (CI). The 25th to 75th percentiles were reported as P25-P75. Total follow-up time was reported as the time of index colonoscopy until first censored time point. Because patients were grouped based on their findings of each of their two consecutive colonoscopies, group allocation was ultimately determined at the time of the second colonoscopy; thus, we also reported a second follow up interval for each of the analyses, which is the time from the date of the second colonoscopy until the first censored time point. All statistical analyses were performed using SPSS version 22 (Armonk, NY: IBM Corp.).

Study Oversight

The Institutional Review Board (IRB) for each of the participating sites approved the creation and analysis of a longitudinal retrospective cohort database of patients with colonic IBD undergoing colonoscopy for CRC surveillance. This study was performed in compliance with the Health Insurance Portability and Accountability Act guidelines.

RESULTS

The baseline characteristics for the 775 patients with longstanding IBD colitis undergoing colonoscopic surveillance meeting initial inclusion criteria (Figure 2) are detailed in Table 1, with characteristics of each of the individual cohorts from the NL, US, and Canada detailed in Supplementary Table 1. The cohort comprised 363 males (47%) with a median age at study entry of 44 (P25-P75: 35-53) years. The most frequent IBD type was UC (n=474; 61%) with median disease duration of 13 (P25-P75: 9-21) years.

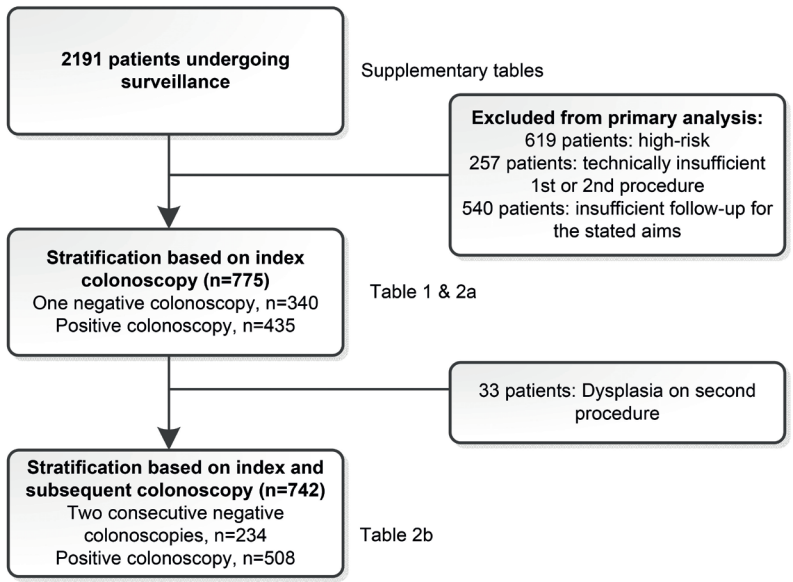


Figure 2. Selection of patients and group allocation

Table 1. Demographic, clinical and endoscopic characteristics of combined cohort (n=775)

Variable	Value
Male	363 (46.8%)
Age at index colonoscopy, years, median (IQR)	44 (35-53)
IBD diagnosis	
- Ulcerative colitis (UC)	474 (61.2%)
- Crohn's colitis (CD)	280 (36.1%)
- Indeterminate colitis (IBD-U)	21 (2.7%)
Disease duration, years, median (IQR)	13 (9-21)
Maximum disease extent (endoscopic)	
- Extensive/pancolitis	390 (50.3%)
- Left-sided/>33% colonic involvement	322 (41.6%)
- Not specified	63 (8.1%)
Medication exposure	
- 5-ASA	658 (84.9%)
- Immunomodulators	386 (52.0%)
- Biologicals	176 (22.7%)
Negative index colonoscopy*	340 (43.9%)
Duration of follow-up after index colonoscopy, years, median (IQR)	6.1 (4.6-8.2)

* defined as a completed surveillance colonoscopy with no post-inflammatory polyps, no strictures, absence of any endoscopic disease activity and no evidence of neoplasia (LGD or higher) in either random or targeted biopsies

The index colonoscopy (i.e. first surveillance colonoscopy within the study period) fulfilled all predefined criteria for a negative exam in 340 patients (44%) with all others classified as positive exams. Both groups were similar in terms of demographics and disease characteristics including age at index colonoscopy, sex, IBD type and disease duration, as well as the number of subsequent surveillance colonoscopies after the index procedure (3.9 vs. 3.9, p=0.76). In general, medication use was higher in the groups with a positive index colonoscopy. The interval between the index colonoscopy and the subsequent surveillance exam was 2.2 years for both groups (p=0.66). Additional characteristics of the two groups stratified according to the findings on the index procedure are detailed in Table 2a. The duration of follow-up for the cohort following the index surveillance procedure was 6.1 (P25-P75: 4.6-8.2) years.

Table 2. Baseline characteristics after stratification based on first (2a) and second (2b) surveillance colonoscopies (after exclusion of patients with a finding of LGD on the second procedure)

	2a			2b		
	Negative index colonoscopy (n=340)	Positive index colonoscopy (n=435)	p-value	2x negative (n=234)	Any positive colonoscopy (n=508)	p-value
Male (%)	151 (44.4%)	212 (48.7%)	0.23	100 (42.7%)	247 (48.6%)	0.14
Age at index colonoscopy, years, median (IQR)	45 (36-53)	43 (33-53)	0.17	46 (37-54)	44 (34-53)	0.12
IBD diagnosis **- Ulcerative colitis** **- Crohn's colitis** **- Indeterminate colitis**	206 (60.6%) 123 (36.2%) 11 (3.2%)	268 (61.6%) 157 (36.1%) 10 (2.3%)	0.72	140 (59.8%) 87 (37.2%) 7 (3.0%)	314 (61.8%) 180 (35.4%) 14 (2.8%)	0.87
Disease duration at index colonoscopy, median (IQR)	14 (9-22)	13 (9-20)	0.33	14 (9-23)	13 (9-20)	0.29
Medication Exposure **- 5-ASA use** **- Immunomodulator use** **- Biological use**	278 (81.8%) 160 (47.1%) 54 (15.9%)	380 (87.4%) 239 (54.9%) 122 (28.0%)	0.03 0.03 <0.01	190 (81.2%) 110 (47.0%) 33 (14.1%)	439 (86.4%) 276 (54.3%) 137 (27.0%)	0.07 0.06 <0.01
Postinflammatory polyps	-	176 (40.5%)	-	-	167 (32.9%)	-
Endoscopic inflammation **- Remission/Inactive** **- Mild** **- Moderate** **- Severe**	340 (100%) - - -	117 (26.9%) 238 (54.7%) 63 (14.5%) 17 (3.9%)	-	234 (100%) - - -	202 (39.8%) 231 (45.5%) 59 (11.6%) 16 (3.1%)	-

Table 2. Continued

	2a			2b		
	Negative index colonoscopy (n=340)	Positive index colonoscopy (n=435)	p-value	2x negative (n=234)	Any positive colonoscopy (n=508)	p-value
Duration of follow-up, median -after index colonoscopy - after second colonoscopy	6.3 (4.8-8.6) 3.8 (2.2-5.4)	6.1 (4.4-8.1) 3.5 (2.1-5.3)	0.12 0.24	6.3 (4.9-8.7) 4.0 (2.3-5.6)	6.0 (4.4-8.1) 3.4 (2.1-5.3)	0.05 <0.01
Total colonoscopies during follow-up	3.9	3.9	0.76	3.9	4.0	0.71
Median time between index and second colonoscopy	2.2 (1.9-3.1)	2.2 (1.6-3.0)	0.66	2.2 (1.9-3.1)	2.2 (1.7-3.0)	0.94

Primary Outcome: Risk of aCRN according to the index and subsequent surveillance colonoscopy findings

The frequency of aCRN cases on follow-up after a negative index colonoscopy was 1/340 (0.3%) compared to 12/435 (2.8%) in those with a positive index colonoscopy. Patients were further stratified according to the findings on the subsequent surveillance colonoscopy (Figure 3).The incidence rate of aCRN in those with double negative surveillance exams was 0/100pty compared to 0.29-0.76/100pty in those with at least one positive exam (p=0.01, log-rank test, Figure 4). That is, no cases of aCRN developed over the follow-up period in those patients with a negative index colonoscopy and negative subsequent surveillance colonoscopy (NEG-NEG). Incidence for the subgroups were: NEG-POS 0.29/100pty (95% CI, 0.01-1.41), POS-NEG 0.43/100pty (95% CI, 0.07-1.43) and POS-POS 0.76/100pty (95% CI, 0.37-1.39). The time interval between the index surveillance colonoscopy and subsequent surveillance exam was the same between both the double negative group and the group with at least one positive colonoscopy (median 2.2 years), as was the number of surveillance colonoscopies performed during the follow-up period. The frequency of colectomies for the subgroups (NEG-NEG; NEG-POS; POS-NEG; POS-POS) were 8/234 (3.4%), 6/93 (6.5%), 5/113 (4.4%) and 18/302 (6.0%), respectively (p=0.51). The total follow up time following the second surveillance procedure for the cohort was 3.6 years.

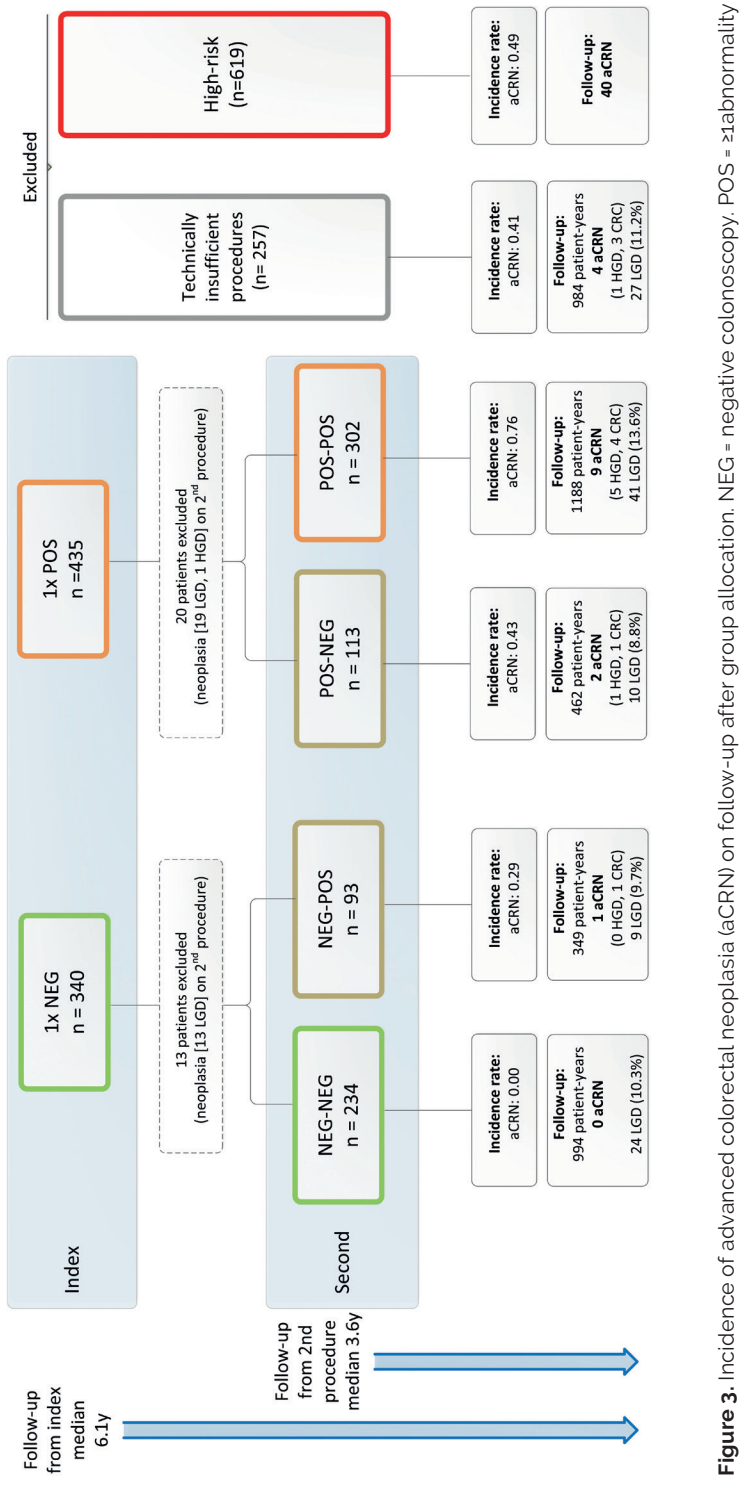


Figure 3. Incidence of advanced colorectal neoplasia (aCRN) on follow-up after group allocation. NEG = negative colonoscopy. POS = ≥1abnormality on colonoscopy.

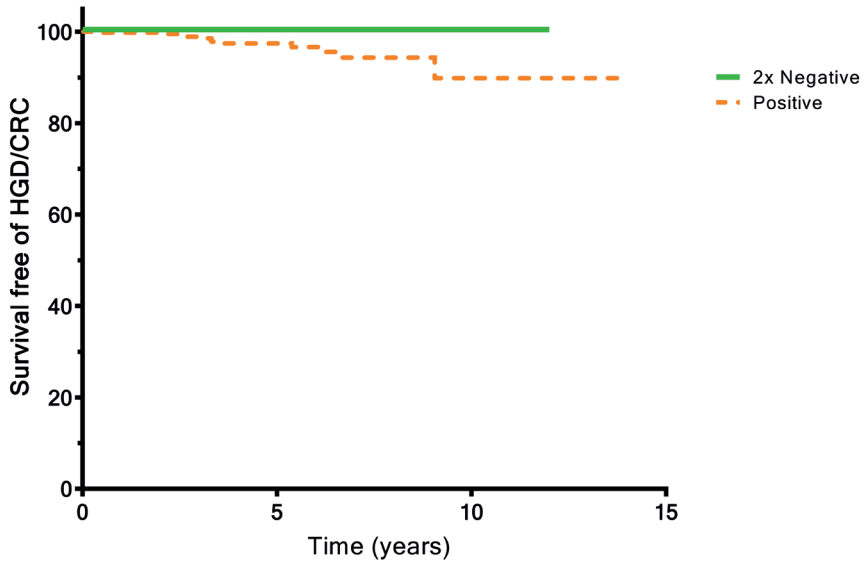


Figure 4. Rate of aCRN according to consecutive surveillance procedure findings (double negative versus at least one positive) *Log-rank test, p=0.01.*

Secondary Outcomes:

Of interest, there were 91 additional patients who had 2 consecutive negative colonoscopies at *any* time during the study period but not including the index exam closest to the start of the predetermined study period (for example, a patient with a positive colonoscopy in 2000 at enrollment (POS index colonoscopy) who had negative examinations according to the predefined criteria in 2005 and 2007). Importantly, none of these patients developed aCRN over the period of follow-up (data not shown).

Among patients in whom the index and/or subsequent surveillance procedure was technically insufficient (n=257) and thus precluded them from the primary analysis, 4 cases of aCRN occurred during the follow-up period, with an incidence rate of 0.41/100pty.

All four groups (NEG-NEG; NEG-POS; POS-NEG; POS-POS) were found to have LGD during subsequent follow-up, but there were no significant differences between the groups with respect to frequency and incidence rate (p=0.68). (Figure 3).

Inclusion of high-risk patients

We performed a sensitivity analysis in which we included the 619 patients who were excluded from the primary analysis at the outset of the study because of pre-defined high-risk features, i.e. PSC, history of any dysplasia prior to, or at, index colonoscopy, prior history of stricture, or first-degree relative with a history of CRC (Supplementary tables 2&3) The overall rate of aCRN in this extended cohort over the follow-up period was 0.49/100pty. Although the overall incidence rates for aCRN were higher compared to the selected cohort for the primary analysis, the previously observed differences in incidence rates remained significantly different. (Supplemental figure 1) The incidence rates for the subgroups were: NEG-NEG 0.15/100pty (95% CI, 0.03-0.51), NEG-ABN 0.44/100pty (95% CI, 0.07-1.45), ABN-NEG 0.65/100pty (95% CI, 0.21-1.56), ABN-ABN 0.73 (95% CI, 0.38-1.27). As expected, those in this extended group who were allocated to the double negative group based on two consecutive negative surveillance colonoscopies had the lowest incidence of aCRN when compared to those with either exam labeled positive, albeit not 0/100pty as in the primary analysis that excluded this "high-risk" cohort.

DISCUSSION

In this well-characterized, multinational and multicenter database of patients with longstanding IBD colitis undergoing routine CRC surveillance, we found markedly low rates of subsequent aCRN diagnoses in patients with a negative index exam according to predefined criteria. The incidence rate of aCRN after a single negative surveillance colonoscopy was already low at 0.09/100 pty, and this rate dropped to zero in those patients with two consecutive negative surveillance exams during the follow-up period. Moreover, this pattern and its statistical significance were also seen when we analyzed the extended cohort that included patients with any high-risk characteristics for aCRN (e.g. PSC, history of any neoplasia prior to, or at, index colonoscopy, prior history of stricture, or first-degree relative with a history of CRC). Our findings suggest that, at least in the population with longstanding IBD colitis and otherwise no additional high-risk features, a CRC surveillance interval longer than 2

years is safe following two negative colonoscopies. Our predetermined definition of a negative colonoscopy was strict, yet it was based on readily available parameters recommended in current guidelines. [3,12]

While patients with IBD colitis are considered to carry a higher than average CRC risk, estimated at 5% after 20 years disease duration[2], the appropriateness of the recommended intervals of colonoscopic surveillance in this population has been incompletely investigated. Considering that the overall risk of CRC in IBD is decreasing over time [2,13] and the sensitivity for detecting dysplasia is increasing due in part to technological advancements in neoplasia detection [14], if surveillance is maintained at overly frequent intervals in all patients with IBD, IBD surveillance programs are at risk of becoming high-intensity/low-value cancer prevention strategies. European guidelines advocate a risk stratification model when determining appropriate surveillance intervals, but recommendations are inconsistent geographically and the data corroborating such recommendations are limited.[3,5,15] Data on incidence, test sensitivity, natural history and sojourn time of precursor lesions are essential to optimize the current surveillance strategies.

We designed our risk stratification model for the present study according to current British and European guidelines, which recommend allocating patients undergoing IBD surveillance into one of three groups[3,15] -low, intermediate, and high-risk- each with distinct recommended surveillance intervals. We further made our definition of a "negative" colonoscopy strict in order to truly define a lower risk IBD population, but also to maximize use of objective measures to enhance clinical reproducibility and facility in a practice-based setting. Patient characteristics including PSC[16,17], family history of CRC[18] and personal history of neoplasia [19], as well as endoscopic features including strictures[20], inflammation[21,22], and post-inflammatory polyps[20,23,24] have all been shown to increase the risk of subsequent neoplastic transformation in IBD colitis; thus, complete absence of these features was necessary to meet criteria for a negative investigation. Based on these criteria, less than 30% of patients in our database had high-risk demographics that necessitated yearly surveillance colonoscopies. That said, the risk associated with post-inflammatory polyps may

vary according to the density and extent of post-inflammatory polyps and depends ultimately on how completely surveillance can still be performed. More research is required to understand the true risk that post-inflammatory polyps pose, be it direct (by neoplastic transformation of the polyp itself) or indirect (due to hindered visualization of other neoplastic polyps). While there are emerging data suggesting that post-inflammatory polyps are not independently associated with an increased risk of neoplasia, we chose to err on the side of conservative and categorized an endoscopic finding of post-inflammatory polyps as a positive colonoscopy. Among the remaining low-risk patients, over one-third had at least one negative surveillance colonoscopy and underscores the potential cost- and resource-saving, not to mention patient convenience implications if surveillance intensity can be downgraded for this select population.

The definition of a low-risk surveillance population for risk stratification purposes in our study also has biological credence. Patients without signs of previous or ongoing inflammation have acquired less cumulative inflammatory damage over time. A decreased mutational burden translates into a decreased risk of developing aCRN. [25] Moreover, endoscopically active disease, strictures and post-inflammatory polyps all affect visibility during colonoscopy and thus the sensitivity of the colonoscopy itself for dysplasia and CRC detection. Having two strictly defined negative consecutive colonoscopies with enhanced diagnostic sensitivity broadly reduces the likelihood of missed dysplasia and optimizes the negative predictive value of colonoscopy in clinical practice.

While our data raise the questions of whether 1) we may rely on the ability of two consecutive negative surveillance colonoscopies to adequately predict *sustained* low aCRN risk and, accordingly, 2) whether we may safely prolong surveillance intervals, it is important to bear two caveats in mind when interpreting our data. Firstly, although we attempted to maximize follow-up times, we had insufficient data to look at the aCRN risk beyond 5 years. Secondly, in the subgroup of patients with two consecutive negative colonoscopies, LGD was nonetheless detected in 11% of patients during follow-up, thus implying that there is still a role for surveillance in these patients. That

said, having *any* two consecutive negative surveillance examinations, irrespective of the findings on index examination, predicted an aCRN rate of only 0.15/100pty.

As a contextual comparison, in the non-IBD background population, the lifetime risk of developing CRC is approximately 4.5%, and is subject to regional and temporal variations.[26] For IBD patients, the risk of CRC is broadly estimated to be increased by a factor of 2, although numbers vary according to the population studied.[2,27] Nevertheless, caution should be exercised when comparing neoplasia outcomes between IBD and non-IBD patients, particularly since the trajectory of dysplasia progression is, generally speaking, more rapid in the former and implicates nuances in the dysplasia-carcinoma sequence.[19] Indeed, CRC screening intervals for non-IBD patients may safely extend up to 10 years, far more prolonged than the currently recommended screening intervals in longstanding IBD colitis patients. [8,28,29]

Our study has some limitations, in addition to those inherent to any retrospective analysis. First, although our duration of follow-up was sufficient for achieving our study aims, we are unable to reliably comment on the risk of aCRN after 5 years. Secondly, the index colonoscopy was defined as the first surveillance exam within our predefined study period and thus may not represent the true index surveillance colonoscopy in patients' IBD courses. That said, this may better represent the real-world scenario and enhance the applicability of our findings, since two negative consecutive colonoscopies may occur at any point during patients' surveillance program. Thirdly, because our database was strictly limited to surveillance colonoscopies, we did not specifically investigate whether intermittent flares between surveillance exams significantly affect the neoplasia risk. Our study was also not designed to comment on the optimal screening interval for this low-risk group, nor of the necessity of concomitant clinical remission. Lastly, because chromoendoscopy was not routinely available at the included institutions for most of the included study period, we were unable to evaluate its adjunctive role in defining a negative colonoscopy and subsequent neoplastic risk.

There are several strengths to our study. By predefining variables and strict selection criteria, we were able to combine three large cohorts of patients with confirmed

IBD colitis from three different countries undergoing CRC surveillance without compromising detailed documentation of patients' baseline demographics, disease-related characteristics, and follow-up colonoscopic and histologic findings. In this way, we maximized generalizability, sample size, and power, while still precisely and reliably categorized patients according to our predetermined criteria.

In conclusion, in this large multicenter, multinational cohort of well-characterized patients with IBD colitis undergoing active CRC surveillance, we identified that in a selected low-risk group, having two consecutive negative surveillance exams predicts a very low and potentially negligible risk of aCRN on continued follow-up. Our findings support the safety of intervals greater than two years for these low-risk patients. While we believe these patients can be safely surveyed at a 5-year interval, the robustness of this recommendation would need to be tested in further longitudinal research.

REFERENCES

1. Beaugerie, L. *et al.* Risk of colorectal high-grade dysplasia and cancer in a prospective observational cohort of patients with inflammatory bowel disease. *Gastroenterology* **145,** 166–175.e8 (2013).
2. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
3. Cairns, S. R. *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* **59,** 666–689 (2010).
4. Annese, V. *et al.* European evidence based consensus for endoscopy in inflammatory bowel disease. *J. Crohns. Colitis* **7,** 982–1018 (2013).
5. Farraye, F. A. *et al.* AGA medical position statement on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* **138,** 738–45 (2010).
6. Pinsky, P. F. *et al.* The yield of surveillance colonoscopy by adenoma history and time to examination. *Clin. Gastroenterol. Hepatol.* **7,** 86–92 (2009).
7. Robertson, D. J. *et al.* Using the results of a baseline and a surveillance colonoscopy to predict recurrent adenomas with high-risk characteristics. *Ann. Intern. Med.* **151,** 103–9 (2009).
8. Brenner, H. *et al.* Low risk of colorectal cancer and advanced adenomas more than 10 years after negative colonoscopy. *Gastroenterology* **138,** 870–6 (2010).
9. Singh, H., Turner, D., Xue, L., Targownik, L. E. & Bernstein, C. N. Risk of developing colorectal cancer following a negative colonoscopy examination: evidence for a 10-year interval between colonoscopies. *JAMA* **295,** 2366–73 (2006).
10. Rex, D. K., Ponugoti, P. L., Johnson, C. S., Kittner, L. & Yanda, R. J. Neoplasia at 10-year follow-up screening colonoscopy in a private U.S. practice: comparison of yield to first-time examinations. *Gastrointest. Endosc.* (2017). doi:10.1016/j.gie.2017.04.035
11. Atkin, W. S., Saunders, B. P., British Society for Gastroenterology & Association of Coloproctology for Great Britain and Ireland. Surveillance guidelines after removal of colorectal adenomatous polyps. *Gut* **51 Suppl 5,** V6-9 (2002).
12. Magro, F. *et al.* Third European Evidence-Based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J. Crohn's Colitis* (2017). doi:10.1093/ecco-jcc/jjx008
13. Kappelman, M. D. *et al.* Risk of Cancer in Patients With Inflammatory Bowel Diseases: A?Nationwide Population-based Cohort Study With 30 Years of Follow-up Evaluation. *Clin. Gastroenterol. Hepatol.* **12,** 265–273.e1 (2014).

14. Carballal, S. *et al.* Real-life chromoendoscopy for neoplasia detection and characterisation in long-standing IBD. *Gut* gutjnl-2016-312332 (2016). doi:10.1136/gutjnl-2016-312332
15. Magro, F. *et al.* Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J. Crohn's Colitis* **11,** 649–670 (2017).
16. Claessen, M. M. H., Vleggaar, F. P., Tytgat, K. M. A. J., Siersema, P. D. & van Buuren, H. R. High lifetime risk of cancer in primary sclerosing cholangitis. *J. Hepatol.* **50,** 158–64 (2009).
17. Torres, J., Pineton de Chambrun, G., Itzkowitz, S., Sachar, D. B. & Colombel, J.-F. Review article: colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **34,** 497–508 (2011).
18. Askling, J. *et al.* Family history as a risk factor for colorectal cancer in inflammatory bowel disease. *Gastroenterology* **120,** 1356–62 (2001).
19. Fumery, M. *et al.* Incidence, Risk Factors, and Outcomes of Colorectal Cancer in Patients with Ulcerative Colitis with Low-Grade Dysplasia: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* (2016). doi:10.1016/j.cgh.2016.11.025
20. Rutter, M. D. *et al.* Cancer surveillance in longstanding ulcerative colitis: endoscopic appearances help predict cancer risk. *Gut* **53,** 1813–1816 (2004).
21. Gupta, R. B. *et al.* Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* **133,** 1099-105–1 (2007).
22. Rutter, M. *et al.* Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* **126,** 451–9 (2004).
23. Velayos, F. S. *et al.* Predictive and protective factors associated with colorectal cancer in ulcerative colitis: A case-control study. *Gastroenterology* **130,** 1941–9 (2006).
24. Lutgens, M. *et al.* A rule for determining risk of colorectal cancer in patients with inflammatory bowel disease. *Clin. Gastroenterol. Hepatol.* **13,** 148–54.e1 (2015).
25. Flores, B. M., O´Connor, A. & Moss, A. C. Impact of Mucosal Inflammation on Risk of Colorectal Neoplasia in Patients with Ulcerative Colitis: A Systematic Review and Meta-Analysis. *Gastrointest. Endosc.* (2017). doi:10.1016/j.gie.2017.07.028
26. Siegel, R. L. *et al.* Colorectal cancer statistics, 2017. *CA. Cancer J. Clin.* **67,** 177–193 (2017).
27. Nguyen, G. C. & Bressler, B. A tale of two cohorts: are we overestimating the risk of colorectal cancer in inflammatory bowel disease? *Gastroenterology* **143,** 288–90 (2012).
28. Singh, H., Nugent, Z., Mahmud, S. M., Demers, A. A. & Bernstein, C. N. Predictors of Colorectal Cancer After Negative Colonoscopy: A Population-Based Study. *Am. J. Gastroenterol.* **105,** 663–673 (2010).

29. Lieberman, D. A. *et al.* Low rate of large polyps (>9 mm) within 10 years after an adequate baseline colonoscopy with no polyps. *Gastroenterology* **147,** 343–50 (2014).

SUPPLEMENTARY MATERIALS

Supplementary table 1. Database characteristics for primary analysis groups: comparison NL-US-CANADA

	Netherlands (n=483)	US (n=201)	Canada (n=91)
Male (%)	228 (47.2%)	94 (46.8%)	41 (45.1%)
Age at study inclusion, median (IQR)	47 (39-54)	40 (30-51)	40 (31-49)
IBD diagnosis - Ulcerative colitis - Crohn's colitis - Indeterminate colitis	295 (61.1%) 179 (37.1%) 9 (1.9%)	95 (47.3%) 98 (48.8%) 8 (4.0%)	84 (92.3%) 3 (3.3%) 4 (4.4%)
Maximum disease extent - Extensive/pancolitis - Left-sided/>33% colonic involvement - Unknown	257 (53.2%) 188 (38.9%) 38 (7.9%)	92 (45.8%) 88 (43.8%) 21 (10.4%)	41 (45.1%) 46 (50.5%) 4 (4.4%)
Medication use - 5-ASA - Thiopurines - Biologicals	398 (82.4%) 214 (44.3%) 51 (10.6%)	172 (85.6%) 131 (65.2%) 110 (54.7%)	88 (96.7%) 54 (59.3%) 15 (16.5%)
Total surveillance colonoscopies per patient	3.9	3.8	4.5
Median time between index and second colonoscopy	2.5 (2.0-3.2)	1.5 (1.0-2.3)	2.9 (1.1-4.4)

Supplementary table 2. Overview of the cohort including high risk patients (n=1093)

Variable	Value
Male (%)	564 (51.6%)
Age at index colonoscopy, median	44 (34-53)
IBD diagnosis - Ulcerative colitis - Crohn's colitis - Indeterminate colitis	697 (63.8%) 362 (33.1%) 34 (3.1%)
Maximum disease extent (endoscopic) - Extensive/pancolitis - Left-sided/>33% colonic involvement - Not specified	567 (51.9%) 420 (38.4%) 106 (9.7%)
PSC	153 (14.0%)

Table S2. Continued

Variable	Value
Medication use	
- 5-ASA	905 (82.8%)
- Thiopurines	524 (47.9%)
- Biologicals	226 (20.7%)
Negative index colonoscopy	443 (40.5%)
Duration of follow-up after index colonoscopy, median	6.0 (4.3-8.1)
High-risk demographics*	318 (29.1%)

*at least one of the following: PSC, stricture, family history of CRC, any dysplasia before surveillance, any dysplasia on first surveillance procedure. Any patient with CRC prior to enrollment was not included in the database

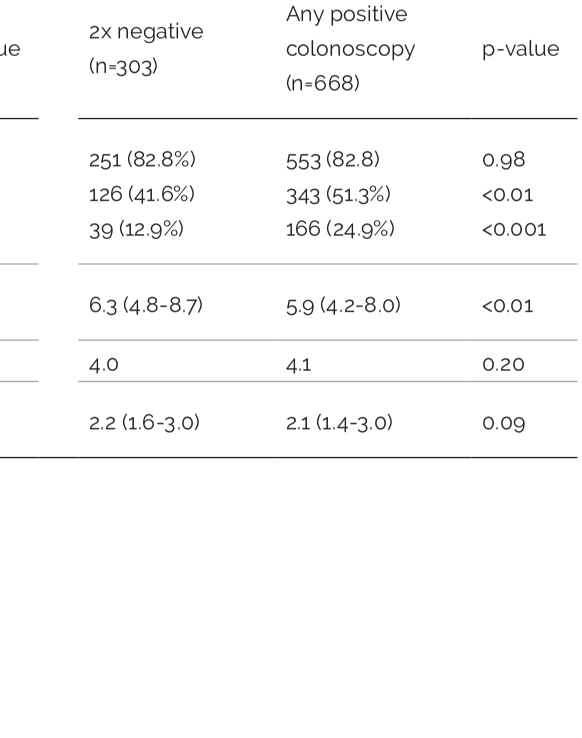
Supplementary table 3. Baseline characteristics after stratification based on first and second surveillance colonoscopies (including high-risk patients)

	Negative index colonoscopy (n=443)	Positive index colonoscopy (n=650)	p-value	2x negative (n=303)	Any positive colonoscopy (n=668)	p-value
Male (%)	223 (50.3%)	341 (52.6%)	0.21	146 (48.2%)	349 (52.2%)	0.24
Age at index colonoscopy, median (IQR)	43 (33-53)	45 (34-53)	0.64	46 (36-53)	42 (33-52)	<0.01
IBD diagnosis **- Ulcerative colitis** **- Crohn's colitis** **- Indeterminate colitis**	286 (64.6%) 143 (32.3%) 14 (3.2%)	411 (63.2%) 219 (33.7%) 20 (3.1%)	0.89	195 (64.4%) 100 (33.0%) 8 (2.6%)	418 (62.6%) 228 (34.1%) 22 (3.3%)	0.79
Disease duration at index colonoscopy, median (IQR)	13 (9-20)	14 (9-22)	0.37	14 (9-23)	13 (9-20)	0.10
PSC	68 (15.3%)	85 (13.1%)	0.29	45 (14.9%)	94 (14.1%)	0.75
Post-inflammatory polyps	-	223 (34.3%)	-	-	200 (29.9%)	-
Endoscopic inflammation (index) **- No activity** **- Mild** **- Moderate** **- Severe**	443 (100%) - - -	206 (31.7%) 338 (52.0%) 87 (13.4%) 19 (2.9%)	-	303 (100%) - - -	263 (39.4%) 311 (46.6%) 77 (11.5%) 17 (2.5%)	-

Table S3: Continued

	Negative index colonoscopy (n=443)	Positive index colonoscopy (n=650)	p-value	2x negative (n=303)	Any positive colonoscopy (n=668)	p-value
Medication Exposure **- 5-ASA** **- Thiopurines** **- Biologicals**	368 (83.1%) 195 (44.0%) 66 (14.9%)	537 (82.6%) 329 (50.6%) 160 (24.6%)	0.85 0.03 <0.01	251 (82.8%) 126 (41.6%) 39 (12.9%)	553 (82.8) 343 (51.3%) 166 (24.9%)	0.98 <0.01 <0.001
Duration of follow-up after index colonoscopy, median	5.9 (4.3-7.9)	6.2 (4.5-8.6)	0.03	6.3 (4.8-8.7)	5.9 (4.2-8.0)	<0.01
Number of colonoscopies	4.1	4.0	0.77	4.0	4.1	0.20
Median time between index and second colonoscopy	2.1 (1.3-3.0)	2.1 (1.6-3.0)	0.13	2.2 (1.6-3.0)	2.1 (1.4-3.0)	0.09

Consecutive negative findings on surveillance colonoscopies



Supplementary figure 1. Rate of aCRN according to consecutive surveillance procedure findings (double negative versus at least one positive) for the full cohort. Log-rank test, p=0.03.

CHAPTER 5

Malignant and Non-malignant Complications of the Rectal Stump in Patients with Inflammatory Bowel Disease

Inflammatory Bowel Diseases, 2019

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ABSTRACT

Background: Patients with refractory inflammatory bowel disease (IBD) might require a subtotal colectomy with construction of an ileostomy. Due to the risk of nerve damage and pelvic sepsis, the diverted rectum is often left in situ. Evidence on long-term complications of this rectal stump is limited, particularly in patients with Crohn's disease (CD). In addition to the risk of development of neoplasia, diversion proctitis is a frequently reported rectal stump associated complication. Surprisingly, clear recommendations concerning rectal stump surveillance and timing of proctectomy are lacking.

Methods: Through the use of a pathology database and a review of medical records, we established a cohort of IBD patients with a diverted rectum. Among these patients, long-term complications of the rectal stump were identified. Main endpoint was advanced neoplasia (carcinoma or high-grade dysplasia [HGD]) in the rectal stump. Risk factors for advanced neoplasia were identified using Cox regression modeling. In the second, prospective part of the study, a questionnaire was sent out to 165 patients with either a rectal stump in situ or who had undergone a proctectomy, in order to identify differences in patient-reported outcome measures associated with the excision of the rectal stump.

Results: From 530 patients with IBD and a (temporal) diversion of the rectum, we included 250 patients in whom the rectal stump was left in situ for more than 12 months. The majority of patients was female (61%) and had Crohn's disease (67%). On follow-up (median 8 years), 8 carcinomas, 2 cases of high-grade dysplasia and 7 cases of low-grade dysplasia were found with incidence rates of 3.9 and 8.5 per 1000 patient-years of follow-up, for cancer and all neoplasia, respectively. The 8 cases of rectal stump cancer (RSC) were diagnosed after a median of 15 years after colectomy. A history of colorectal neoplasia was associated with advanced rectal stump neoplasia. Out of 191 patients with endoscopic follow-up, rectal stump inflammation occurred in 161 (88.5%) patients. Results of the questionnaire did not show a significant difference in quality of life between patients with and patients without a rectal stump, although the latter group reported significantly more sexual and urinary symptoms than patients with a rectal stump in situ. The majority of rectal stump patients reported rectal blood loss, but 65.5% of them were not or barely limited in daily life by their rectal stumprelated problems.

Conclusion: RSC has a low incidence rate, with patients with a history of colonic neoplasia carrying the highest risk of developing this severe complication. We observed no significant differences in quality of life between rectal stump and post-proctectomy patients, but proctectomy surgery is associated with sexual and urinary complications.

INTRODUCTION

Nearly one third and more than 70% of the patients diagnosed with ulcerative colitis (UC) and Crohn's disease (CD) respectively, will require intestinal surgery at some point in the course of their disease.[1,2] In the setting of refractory severe colitis, a subtotal colectomy with an ileostomy is the procedure of choice. Typically, in UC the preferred restoration technique is a proctectomy in combination with an ileal pouch-anal anastomosis (IPAA).[3] When pouch surgery is not appropriate or contraindicated, an ileostomy can be a definitive outcome. Due to the potential risk of pelvic nerve damage and pelvic septic complications, the rectum may be left in situ. Likewise, in 25% of patients with colonic CD an end ileostomy is constructed with a closed rectal stump.[4] In total, 12.8 to 36.7% of UC and CD patients are estimated to end up with a diverted rectum after subtotal colectomy.[5,6]

Data on surgery-related morbidity and long-term outcomes of the rectal stump are mostly derived from small retrospective studies, precluding a reliable estimation of the long-term risk of malignant and non-malignant complications.[7] A meta-analysis on malignancies in the rectal stump of IBD patients by Derikx et al., based on 13 single-centered retrospective studies, reported a pooled prevalence of rectal stump carcinoma (RSC) of 2.1% (95% C.I. 1.3-3.0).[8] More recently, Abdalla et al. reported a standardized incidence ratio of 3.8 for RSC in a Swedish nationwide cohort of UC patients with a diverted rectum.[9] In addition to dysplasia and cancer, post-colectomy patients are regularly confronted with "diversion proctitis", a condition characterized by abdominal discomfort and mucopurulent rectal discharge.[10] In a systematic review by Kabir et al. the prevalence of this underreported complication was estimated to be at least 90%.[11]

In order to prevent development of RSC, enrolling these patients in a surveillance program should be considered. At this point, clear guideline recommendations are lacking and surveillance itself is inconsistently performed in daily clinical practice.[12,13]

The primary objective of this study was to assess the incidence rate of dysplasia and carcinoma in the rectal stump of IBD patients and to identify associated risk factors. Secondary objectives were to quantify the non-malignant complications

originating from a diverted rectum and to estimate the quality of life in patients after proctectomy.

METHODS

Study design and study population

We explored the risk of rectal stump neoplasia and non-malignant complications in a retrospective cohort of IBD patients who underwent colonic resections. Additionally, we performed a prospective study to estimate the quality of life and patient reported outcomes, employing a questionnaire sent to IBD patients who had either undergone a proctectomy or with a diverted rectum in situ for more than 12 months.

Retrospective cohort

We identified a cohort of IBD patients with a diverted rectum in a tertiary referral center (University Medical Center Utrecht), using the Dutch nationwide registry of histopathology and cytopathology (PALGA) followed by retrospective analysis of medical records. In order to identify risk factors for advanced neoplasia of the rectal stump in a case-control setting, we extended the PALGA search algorithm to include three other high volume IBD centers (2 secondary centers, 1 tertiary referral center).

The PALGA database contains nationwide coverage of all pathology reports generated since 1991. A PALGA search was accomplished to identify all IBD patients who underwent a partial or subtotal colectomy with the retention of the colorectal stump using an algorithm combining the terms "ulcerative colitis", "Crohn's disease", "indeterminate colitis" and "chronic idiopathic inflammatory bowel disease" in combination with "all resections" of "rectal stump", "rectum" or "colon" between January 1st, 1991 and December 31st, 2015. Every pathology report was manually reviewed to obtain an accurate selection suitable for inclusion. Additionally, we performed a search using the hospital-based coding for IBD to include cases possibly missed by the search algorithm.

In- and exclusion criteria

From this preselection we included patients with ulcerative colitis (UC), Crohn's disease (CD) or indeterminate colitis (IBD-U), who had had a subtotal or partial colectomy with retention of a colorectal stump. Patients who had a diverted rectum for less than 12 months were excluded, as well as patients with continuity of the fecal stream, e.g. following construction of an ileal pouch-anal anastomosis (IPAA) or an ileorectal anastomosis (IRA). Furthermore, patients with Lynch syndrome or familial adenomatous polyposis were excluded.

Demographic, clinical, endoscopic and pathologic variables were retrieved from medical records. Variables included were sex, IBD type, alcohol use, smoking, primary sclerosing cholangitis (PSC), familial colorectal cancer (CRC), prior neoplasia and fistula or perianal disease. Prior colorectal neoplasia was defined as low-grade dysplasia (LGD), high-grade dysplasia (HGD) or colorectal cancer (CRC) detected before subtotal colectomy. Included endoscopic surveillance parameters were severity of inflammation, type of inflammation, observed shortening of the stump and stenosis. The severity of endoscopic inflammation was defined as none-to-mild or moderate-to-severe. Inflammation was categorized as diversion colitis, IBD, both or non-specific. Since no well-defined classification system of rectal stump inflammation exists, the variables were coded based on the overall impression reported in the endoscopy and pathology reports.

Prospective study (Questionnaire)

To compare differences in patient-reported outcome measures (PROMs) associated with the excision of the rectal stump, a questionnaire was sent to all patients fulfilling the inclusion criteria with an active hospital-based coding for IBD. Patients were allocated to one of two groups: patients who underwent a subtotal colectomy and had a rectal stump in situ (rectal stump patients) and patients who had undergone a proctectomy (post-proctectomy patients). Exclusion criteria were: last colorectal surgery <12months ago or limited life expectancy. The survey consisted of the EQ-5D-5L-questionnaire and supplementary questions regarding long-term complications

of rectal surgery. Additionally, specific questions concerning rectal stump-related problems were included. The EQ-5D-5L is a non-disease-specific survey which measures five dimensions of life, namely mobility, self-care, usual activities, pain/discomfort and anxiety/depression.[14] A combination of the scores for different dimensions can be converted to a nationality-validated index ranging from -0.11 to 1.00.[15] A second part of the EQ-5D-5L contains a visual analogue scale (VAS), which can be interpreted as a quantitative measure of health as judged by the individual subjects.[14]

Statistical analysis

Incidences of neoplasia were displayed using Kaplan-Meier curves. Time to event was calculated from the moment of diversion of the rectum to the development of neoplasia or the end of follow-up (last endoscopic stump surveillance or excision of the rectal stump. Univariate and multivariate Cox regression modeling was used to identify factors associated with rectal stump neoplasia. Categorical baseline and outcome data were compared using the χ^2 or Fisher exact test, continuous baseline and outcome data were analyzed using the independent student's T-test or the Mann–Whitney U test.

All analyses were performed in IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA) and GraphPad Prism for Windows version 7.02 (GraphPad Software, La Jolla, CA., USA). A two sided p-value of <0.05 was considered statistically significant.

Ethical considerations

This study was conducted in accordance with the declaration of Helsinki and in accordance to the Dutch Medical Research Involving Human Subjects Act (WMO). The medical ethics committee of the University Medical Center Utrecht approved the study. The search in the Dutch Pathology Registry (PALGA) was approved by the PALGA Privacy Commission and Scientific Council.

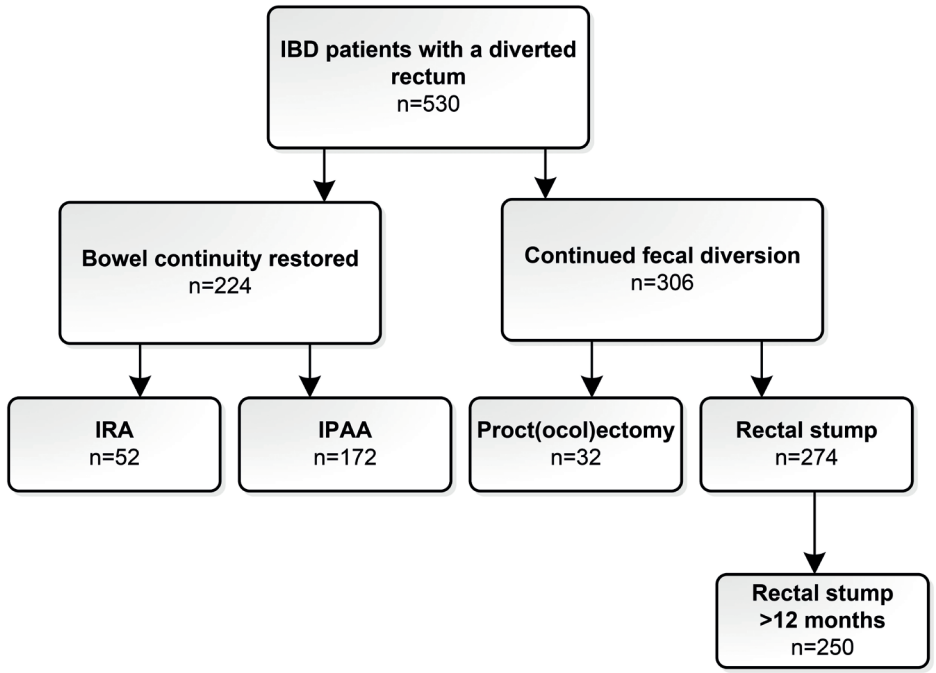


Figure 1. Flowchart of patient selection

RESULTS

Patient selection

In total, 530 unique patients were identified as having IBD and 1 or more colonic resections with a (temporally) diversion of the rectum (Figure 1). The final anatomical situation was ileorectal anastomosis (IRA) in 52 patients (9.8%), ileal pouch-anal anastomosis (IPAA) in 172 patients (32.5%) and a colo- or ileostomy in 306 patients (57.7%). The latter category included 32 patients who underwent a direct proct(ocol) ectomy.

In total, 274 patients were identified with a rectal stump, which was left in situ for more than 12 months in 250 patients. Of this subgroup, 66 (26.4%) patients had UC, 167 (66.8%) had CD and 17 (6.8%) had IBD-U. Ninety-eight patients (39.2%) were male and the mean age at colectomy was 39 (±15) years. Eight patients (3.2%) had a diagnosis of PSC. The most frequent surgical procedures that preceded the diversion of the rectum were a subtotal colectomy (66.4%) and the construction of a colostomy (18.8%).

In 62 out of 250 rectal stump patients (24.8%) a completion proct(ocol)ectomy was eventually performed (ending follow-up). The main reasons for the excision of the colorectal stump were ongoing complaints of bloody rectal discharge (71.0%) and treatment or prevention of cancer (19.4%).

Table 1. Characteristics of patients with a rectal stump for >1 year in situ (n=250)

	n=250
Male sex	98 (39.2%)
IBD subtype	
· CD	167 (66.8%)
· UC	66 (26.4%)
· IBD-U	17 (6.8%)
Age at IBD diagnosis, mean (SD)	31 (15)
PSC	8 (3.2%)
Age at first colonic surgery (leading to fecal diversion)	39 (15)
Type of first surgery (leading to fecal diversion)	
· Subtotal colectomy	166 (66.4%)
· IRA	11 (4.4%)
· Colostomy	47 (18.8%)
· Ileostomy	11 (4.4%)
· Double colostomy	4 (1.6%)
· Double ileostomy	11 (4.4%)
Indication for surgery	
· Refractory disease	206 (82.4%)
· Stenosis	6 (2.4%)
· (risk of) Dysplasia/cancer	6 (2.4%)
· Other	32 (12.8%)
Perianal disease / fistulas	114 (45.6%)
Prior (preoperative) colonic neoplasia	
· All neoplasia	21 (8.4%)
· Advanced neoplasia	5 (2.0%)
Smoking (at time of questionnaire)	
· Yes	33 (13.2%)
· Quit	41 (16.4%)
· No/unknown	176 (70.4%)
Endoscopic surveillance	191 (76.4%)

Follow-up of the rectal stump

In 191 out of 250 patients (76%) with a diverted rectum for more than 1 year, endoscopic follow-up was available (median 8 years, range 1-39).

Table 2. Characteristics of patients with follow-up of the rectal stump (n=191)

Mean follow-up, years (SD)	10.5 (8.8)
Cases of neoplasia	
· LGD	7 (3.7%)
· HGD	2 (1.0%)
· Rectal stump cancer (RSC)	8 (4.2%)
Incidence rate (per 1000 pty)	
· All neoplasia	8.5
· Advanced neoplasia (HGD/CRC)	4.8
· Rectal stump cancer (RSC)	3.9
Rectal stump inflammation	
· No inflammation	21 (11.5%)
· Mild	118 (64.8%)
· Moderate-severe	43 (23.6%)
Proctectomy	62 (24.8%)
Proctectomy indication	
· Neoplasia	11 (4.4%)
· Complaints	44 (17.6%)
· Fear of cancer	1 (0.4%)
· Unknown	7 (2.8%)

Non-malignant complications

In 161 of the 191 patients (84.3%) with follow-up, inflammation of the rectal stump was encountered. From these patients, 115 (71.4%) had diversion colitis and 12 (7.5%) had a combination of diversion colitis and inflammatory bowel disease in the diverted rectum. In a majority of patients, the severity of endoscopically observed inflammation was mild (64.8%). A stenosis of the rectal stump was found in 57 patients, while shortening of the rectal stump was detected in 49 patients.

Rectal stump neoplasia incidence and risk factors

Eight cases of RSC, 2 cases of HGD and 7 cases of LGD occurred in 191 patients. Figure 2 displays Kaplan Meier survival curves for RSC, advanced neoplasia and all neoplasia

(RSC and/or HGD and/or LGD) respectively. Incidence rates were 3.9 and 4.8 per 1000 patient-years of follow-up for RSC and advanced neoplasia (HGD + RSC) respectively. The 8 cases of RSC were diagnosed after a median of 15 years after colectomy (range 2-38 years). In addition, one case of anal squamous dysplasia was identified.

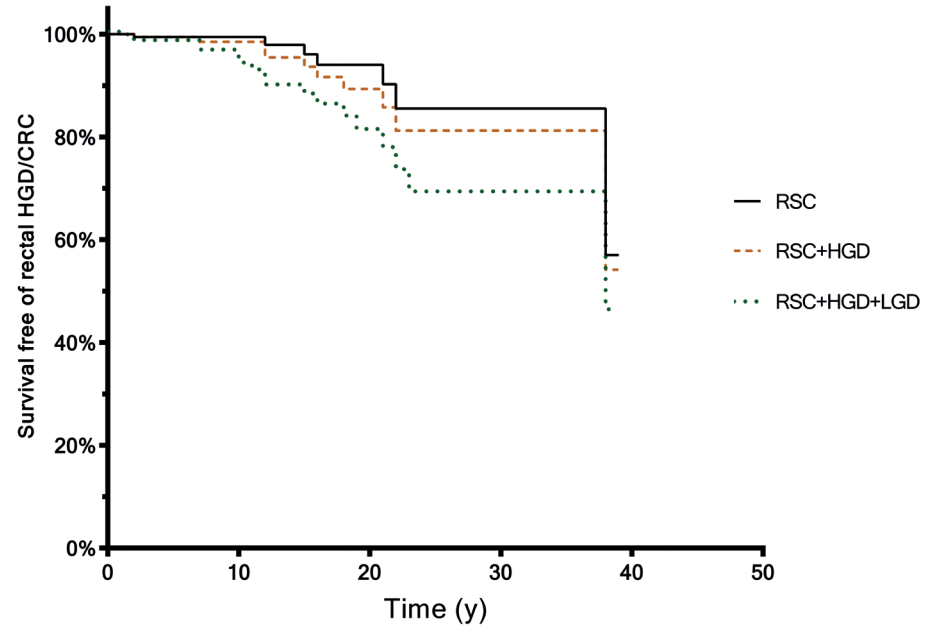


Figure 2. Kaplan-Meier curves displaying survival free rectal stump neoplasia and rectal stump cancer (RSC) in the diverted rectum

In 4 cases, RSC was detected upon endoscopic rectal stump surveillance, In 2 cases,RSC was identified using MRI. Of note, in one MRI-detected case, the RSC was located proximally to a stenosis of the rectal stump. The remaining two cases were detected upon removal of the rectal stump.

After extending the search algorithm to the three other centers and manually reviewing pathology reports, 7 additional cases of advanced rectal stump neoplasia were detected amounting to a total of 17 cases available for the risk factor analysis. Results for Cox-regression analysis into risk factors for advanced rectal stump neoplasia are shown in Table 3. The only factor predictive of advanced neoplasia was a diagnosis of colorectal neoplasia before colectomy (HR 3.8; 95%CI 1.07-13.53).

Limiting the analysis to patients from our referral center (UMCU) did not alter direction or statistical significance of the observed effect (HR 5.2; 95%CI 1.04-26.01). Since no other associations were detected, multivariate analysis was not performed.

Table 3. Univariate Cox-regression analysis for the overall risk of advanced neoplasia in the rectal stump. Additional cases of rectal stump neoplasia included. (HR: hazard ratio)

	HR	95% CI	P-value
Sex (male)	1.479	0.565-3.872	0.425
IBD subtype (CD)	1.282	0.506-3.248	0.600
Age (IBD onset)	1.019	0.981-1.059	0.340
Age (rectal stump)	1.025	0.988-1.063	0.189
Primary Sclerosing Cholangitis	0.045	0.000- >100	0.540
Prior colorectal neoplasia (before colectomy)	3.795	1.065-13.530	0.040
Fistulas	2.192	1.808-5.947	0.123
Rectal stump inflammation (none vs any)	0.366	0.075-1.780	0.213
Known family history of CRC	1.419	0.169-11.883	0.747
Smoking history	0.612	0.123-3.040	0.549

Questionnaire

Baseline characteristics

A total of 105 out of 165 patients responded to our questionnaire (response rate 63.6%). Of these, 61 patients had a rectal stump and 44 patients had undergone a proctectomy (proctocolectomy in one tempo in 12, and in multiple tempi in 32 patients). The final operative status (rectal stump in situ or proctectomy) was established at least 12 months prior to filling out the questionnaire in all patients. The majority of respondents was female and had Crohn's disease.(Table 4) Follow-up time since the last operation was significantly longer for the post-proctectomy group (20 vs 13 years, p=0.013).

Table 4. Characteristics of respondents to questionnaire (n= 105)

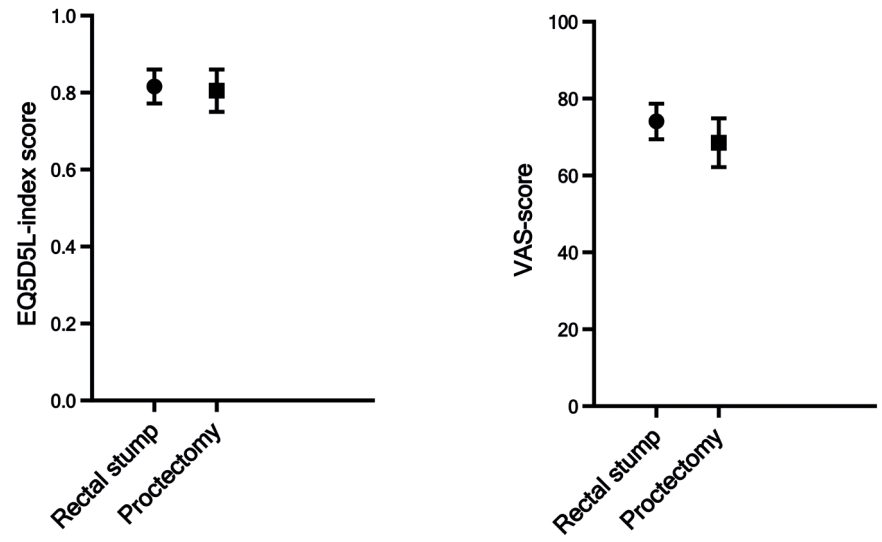
	Rectal stump	Post-proctectomy	p-value
Male sex	26 (42.6%)	17 (38.6%)	0.682
Age, median	53 (16)	59 (14)	0.054
IBD subtype			
· CD	37 (60.7%)	27 (61.4%)	0.853
· UC	18 (29.5%)	14 (31.8%)	
· IBD-U	6 (9.8%)	3 (6.8%)	
Age at IBD diagnosis, mean (SD)	32 (15)	27 (12)	0.101
Years since final operation	13	20	0.013

Health associated quality of life

Both the EQ5D5L-index and VAS-score score did not differ significantly between the rectal stump and the post-proctectomy patients (Table 5). Three different categories of potential complications associated with proctectomy surgery were addressed in the questionnaire: sexual or urinary problems and infertility. The prevalence of sexual difficulties was significantly higher in the post-proctectomy subgroup (p=0.03). Only 7 individuals (15.9%) with an excised rectum reported no sexual problems compared to 18 patients (29.5%) with a rectal stump. Also, the prevalence of urinary complaints differed significantly between the post-proctectomy and rectal stump subgroups (43.2% versus 16.4%, p=0.02). Fertility problems were assessed in females; in 3 respondents (7.0%) with an excised rectum and 1 individual (1.6%) with a rectal stump fertility-related complaints were present (p = 0.19).

Table 5. Subgroup comparison of health associated quality of life values and stump excision associated complications (n/a = not applicable) * = (none/barely) vs (somewhat/quite much/a lot)

	Rectal stump	Post-proctectomy	p-value
EQ5D5L index score, mean	0.82	0.81	0.763
EQ5D5L VAS-score, mean	74.1	68.5	0.148
Sexual difficulties			
· None	18 (29.5%)	7 (15.9%)	
· Barely	13 (21.3%)	4 (9.1%)	
· Somewhat	9 (14.8%)	10 (22.7%)	0.030*
· Quite much	3 (4.9%)	4 (9.1%)	
· A lot	2 (3.3%)	7 (15.7%)	
· n/a	16 (26.2%)	12 (27.3%)	
Urinary difficulties			
· No	50 (82.0%)	25 (56.8%)	
· Yes	10 (16.4%)	19 (43.2%)	0.015
· n/a	1 (1.6%)	-	
Fertility problems (women only)			
· None	27 (44.3%)	19 (43.2%)	
· Yes	1 (1.6%)	3 (7.0%)	0.193
· n/a	33 (54.1%)	21 (47.7%)	



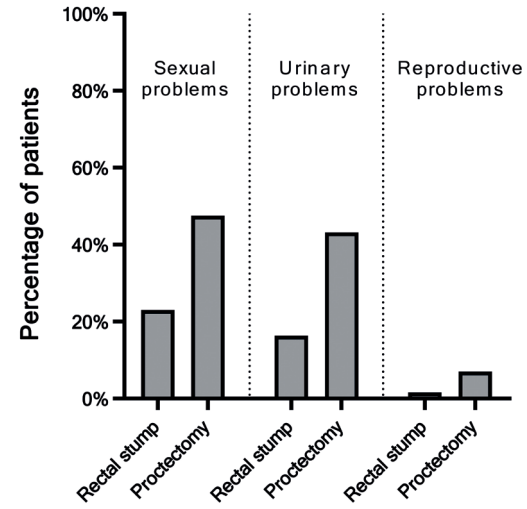


Figure 3. Subgroup comparison of patient reported outcomes; a) EQ5D5L-index scores (mean with 95% CI), b) VAS-score (mean with 95% CI), c) proportion of patients with at least some difficulty or problems in the domains of urinary, sexual and/or reproductive health

Complications of a diverted rectum

Six additional questions regarding rectal stump related problems were included in the questionnaire (Table 6). A majority of patients (83.6%) reported rectal blood loss more than once a week. Sixteen individuals (26.3%) reported rectal pain regularly or often. A minority of patients reported other complaints such as tenesmi, perianal skin problems or an unpleasant odor. Forty patients (65.6%) were not, or only barely restricted in their daily life due to rectal stump-associated complaints. Thirteen persons (21.3%) used some kind of suppository or enema for rectal stump-related problems. Only five patients (8.2%) believed that a proctectomy would improve the quality of their life, 21 respondents (34.4%) judged that the excision of their rectum would not have any positive effect and 35 patients (57.4%) remained undecided.

Table 6. Reported complications of a diverted rectum (n=61)

Blood loss out of the rectal stump	
· Never	10 (16.4%)
· Less than once a week	12 (19.7%)
· Once a week	8 (13.1%)
· More than once a week	15 (24.6%)
· One or more times a day	16 (26.2%)
Rectal pain	
· Never	18 (29.5%)
· Rarely	27 (44.3%)
· Regularly	14 (23.0%)
· Often	2 (3.3%)
Other complaints	
· No other reported problems	43 (70.5%)
· Tenesmus	8 (13.1%)
· Perianal skin problems	4 (6.6%)
· Unpleasant odor	2 (3.3%)
· Fistula	2 (3.3%)
· Ostomy related problems	2 (3.3%)

Table 6. Continued

Limitation of daily life by complaints	
· None	18 (29.5%)
· Barely	22 (36.1%)
· Somewhat	11 (18.0%)
· Quite a lot	3 (4.9%)
· Very much	1 (1.6%)
· n/a	6 (9.8%)
Medical treatment of the rectal stump	
· None	48 (78.7%)
· Suppository/Clyster	13 (21.3%)
Expected improvement in quality of life after proctectomy	
· No	21 (34.4%)
· Yes	5 (8.2%)
· I don't know	35 (57.4%)

DISCUSSION

A high number of both UC and CD patients will end up with a rectal stump after a subtotal colectomy.[5,6] Although research regarding the risk of colon cancer in IBD has generated new insights, data on malignant progression in the rectal stump are still scarce, particularly in patients with CD. In addition to the risk of cancer, a diverted rectum is a major cause of other complaints, such as tenesmus and bloody mucopurulent discharge. The current study was designed to evaluate the major long-term complications of a rectal stump in IBD patients. We found an incidence rate of 4.8 per 1000 patient-years of follow-up for RSC. A history of colorectal neoplasia in the removed colon was associated with development of advanced rectal stump neoplasia. A non-neoplastic condition, rectal stump inflammation predominantly labeled as diversion colitis, was identified in the vast majority of patients with endoscopic follow-up. A completion proctectomy was performed in 25% of patients, most commonly because of the presence of non-malignant stump complications.

The relatively low incidence rate of RSC in our IBD cohort is in agreement with previous reports.[8,9] Our finding that a history of neoplasia seems to be related with advanced rectal stump neoplasia corroborates the finding by Abdalla et al. that prior

severe (high-grade) dysplasia is associated with RSC.[9] This increased risk might be explained by an underlying susceptibility to developing dysplasia, due to genetic background, previous (severe) pancolitis and/or field cancerization.[16] A previously observed association between PSC and rectal stump neoplasia was not replicated in our dataset.[17] Our results support the potential benefit of a low threshold for proctectomy in patients with history of colorectal neoplasia. Aside from the cases of adenomatous dysplasia, one diagnosis of anal squamous dysplasia was found. Reportedly, this neoplastic lesion is human papillomavirus-related, and might therefore be secondary to the use of immunosuppressive drugs.[18]

Although 76% of patients received endoscopic follow-up, there was a wide variation in the duration of follow-up and the length of the surveillance intervals, most likely due to lack of clear guidelines for this category of patients. The high prevalence of diversion colitis in our study is in line with previous reports.[7,11] Of note, there is often great difficulty, both macroscopically and microscopically, in distinguishing diversion colitis from IBD.[10,11,19] Regardless of the fact that rectal stump inflammation is difficult to classify, practically all diverted recta showed redness and a vulnerable mucosa. IBD-related CRC follows an inflammation-dysplasia-carcinoma pathway[17,20], but it is not known whether rectal stump inflammation would accelerate carcinogenesis in a similar way. Unfortunately, this study was not capable of demonstrating an association between severity of rectal stump inflammation and advanced dysplasia or dysplasia in general in the diverted rectum. Since follow-up of the rectal stump was performed in 191 out of 250 patients, our findings cannot be extrapolated to all IBD patients with a rectal stump.

Results of the survey did not show a significant difference between the means of both the EQ5D5L-index and VAS-scores. Interestingly, more than one third of patients with a diverted rectum believed that a proctectomy would not improve their quality of life. Our data showed an association between proctectomy and both urinary and sexual complaints. These results are in line with previous studies on pelvic surgery reporting that an IPAA-procedure is associated with urogenital complications.[21–23] Our questionnaire was not designed to show detailed differences in reproductive

problems, but postponing a rectal excision until reproductive wishes are met may be a safe choice.

As can be expected from the high prevalence of diversion colitis in our cohort, the majority of the included rectal stump patients reported to have rectal blood loss more than once a week. Nonetheless, 65.5% of the surveyed individuals felt not or barely limited in daily life because of their stump-related problems.

Strengths of this study are the large cohort with detailed description of the complex anatomical situations after colorectal surgery for IBD. Second, our cohort consisted of patients with all subtypes of IBD and did not only focus on UC. Furthermore, we enriched our clinical dataset with a self-reported questionnaire to evaluate the personal experiences of the included individuals.

This study has some inherent limitations, due to its retrospective design. For example, no standardized method was used for reporting endoscopic surveillance procedures and there were no definitive criteria used to classify the type and severity of rectal stump inflammation. Furthermore, the main cohort, used for incidence estimations and the survey, originated from a single tertiary center which limits generalizability of the results.

In conclusion, most patients with a rectal stump experience mild complaints, but in a subgroup complications can be severe and might have devastating consequences. The incidence rate of advanced rectal stump neoplasia is low, but the risk of cancer is real. We confirm earlier observations that a history of colorectal neoplasia is associated with malignant progression in the rectal stump. Although inflammation of the diverted rectum is highly prevalent, inflammation of the diverted rectum does not seem to cause significant limitations in daily life. Physicians need to be aware of their patients' surgical anatomy and inform them on the risk of cancer, albeit small, and proctectomy should be discussed. Since proctectomy implies an additional operation with a risk of urogenital complications and since RSC is relatively uncommon, the excision of a diverted rectum should not be performed in the majority of patients. In a

small subgroup of patients, e.g. with a history of colorectal neoplasia or symptomatic diversion colitis, an early proctectomy might be justified.

Practice points:

- The increased risk of colorectal cancer in patients with long-standing and extensive IBD of the colon warrants a surveillance strategy
- Random 4-quadrant biopsy protocols are obsolete, due to a considerably low per-biopsy yield and risk of sampling bias
- Several new techniques may aid the endoscopist in detecting dysplasia during surveillance colonoscopy, thereby increasing the dysplasia yield
- Current guidelines recommend chromoendoscopy with targeted biopsy as the technique of first choice

Research agenda:

- The true impact of surveillance on cancer-related mortality needs to be clarified
- The improvements in dysplasia detection employing high-definition white light endoscopy should be investigated
- The effectiveness of chromoendoscopy needs to be validated in the real life setting
- Clinical trials, preferably using a parallel design, are needed to compare AFI, FICE and i-scan protocols to the methods that are currently in use

REFERENCES

1. Andersson, P. & Soderholm, J. D. Surgery in ulcerative colitis: Indication and timing. in *Digestive Diseases* **27,** 335–340 (2009).
2. Cosnes, J., Gower-Rousseau, C., Seksik, P. & Cortot, A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* **140,** 1785–94 (2011).
3. Parks, A. G., Nicholls, R. J. & Belliveau, P. Proctocolectomy with ileal reservoir and anal anastomosis. *Br. J. Surg.* **67,** 533–8 (1980).
4. Lapidus, A., Bernell, O., Hellers, G. & Löfberg, R. Clinical course of colorectal Crohn's disease: a 35-year follow-up study of 507 patients. *Gastroenterology* **114,** 1151–60 (1998).
5. Böhm, G. & O'Dwyer, S. T. The fate of the rectal stump after subtotal colectomy for ulcerative colitis. *Int. J. Colorectal Dis.* **22,** 277–82 (2007).
6. Harling, H., Hegnhøj, J., Rasmussen, T. N. & Jarnum, S. Fate of the rectum after colectomy and ileostomy for Crohn's colitis. *Dis. Colon Rectum* **34,** 931–5 (1991).
7. Munie, S., Hyman, N. & Osler, T. Fate of the Rectal Stump After Subtotal Colectomy for Ulcerative Colitis in the Era of Ileal Pouch–Anal Anastomosis. *JAMA Surg.* **148,** 408 (2013).
8. Derikx, L. A. A. P., Nissen, L. H. C., Smits, L. J. T., Shen, B. & Hoentjen, F. Risk of Neoplasia After Colectomy in Patients With Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* **14,** 798–806.e20 (2016).
9. Abdalla, M., Landerholm, K., Andersson, P., Andersson, R. E. & Myrelid, P. Risk of Rectal Cancer After Colectomy for Patients With Ulcerative Colitis: A National Cohort Study. *Clin. Gastroenterol. Hepatol.* **15,** 1055–1060.e2 (2017).
10. Wu, X., Liu, X., Katz, S. & Shen, B. Pathogenesis, Diagnosis, and Management of Ulcerative Proctitis, Chronic Radiation Proctopathy, and Diversion Proctitis. *Inflamm. Bowel Dis.* **21,** 703–715 (2015).
11. Kabir, S. I., Kabir, S. A., Richards, R., Ahmed, J. & MacFie, J. Pathophysiology, clinical presentation and management of diversion colitis: a review of current literature. *Int. J. Surg.* **12,** 1088–92 (2014).
12. Magro, F. *et al.* Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J. Crohn's Colitis* **11,** 649–670 (2017).
13. Cairns, S. R. *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* **59,** 666–689 (2010).
14. Herdman, M. *et al.* Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual. Life Res.* **20,** 1727–36 (2011).

15. M Versteegh, M. *et al.* Dutch Tariff for the Five-Level Version of EQ-5D. *Value Health* **19,** 343–52 (2016).

16. Galandiuk, S. *et al.* Field cancerization in the intestinal epithelium of patients with Crohn's ileocolitis. *Gastroenterology* **142,** 855–864.e8 (2012).

17. Lutgens, M. W. M. D. *et al.* Risk Factors for Rectal Stump Cancer in Inflammatory Bowel Disease. *Dis. Colon Rectum* **55,** 191–196 (2012).

18. Wisniewski, A. *et al.* Anal Neoplasia in Inflammatory Bowel Disease: Classification Proposal, Epidemiology, Carcinogenesis, and Risk Management Perspectives. *J. Crohns. Colitis* **11,** 1011–1018 (2017).

19. Asplund, S., Gramlich, T., Fazio, V. & Petras, R. Histologic changes in defunctioned rectums in patients with inflammatory bowel disease: a clinicopathologic study of 82 patients with long-term follow-up. *Dis. Colon Rectum* **45,** 1206–13 (2002).

20. Itzkowitz, S. H. & Yio, X. Inflammation and Cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *AJP Gastrointest. Liver Physiol.* **287,** G7–G17 (2004).

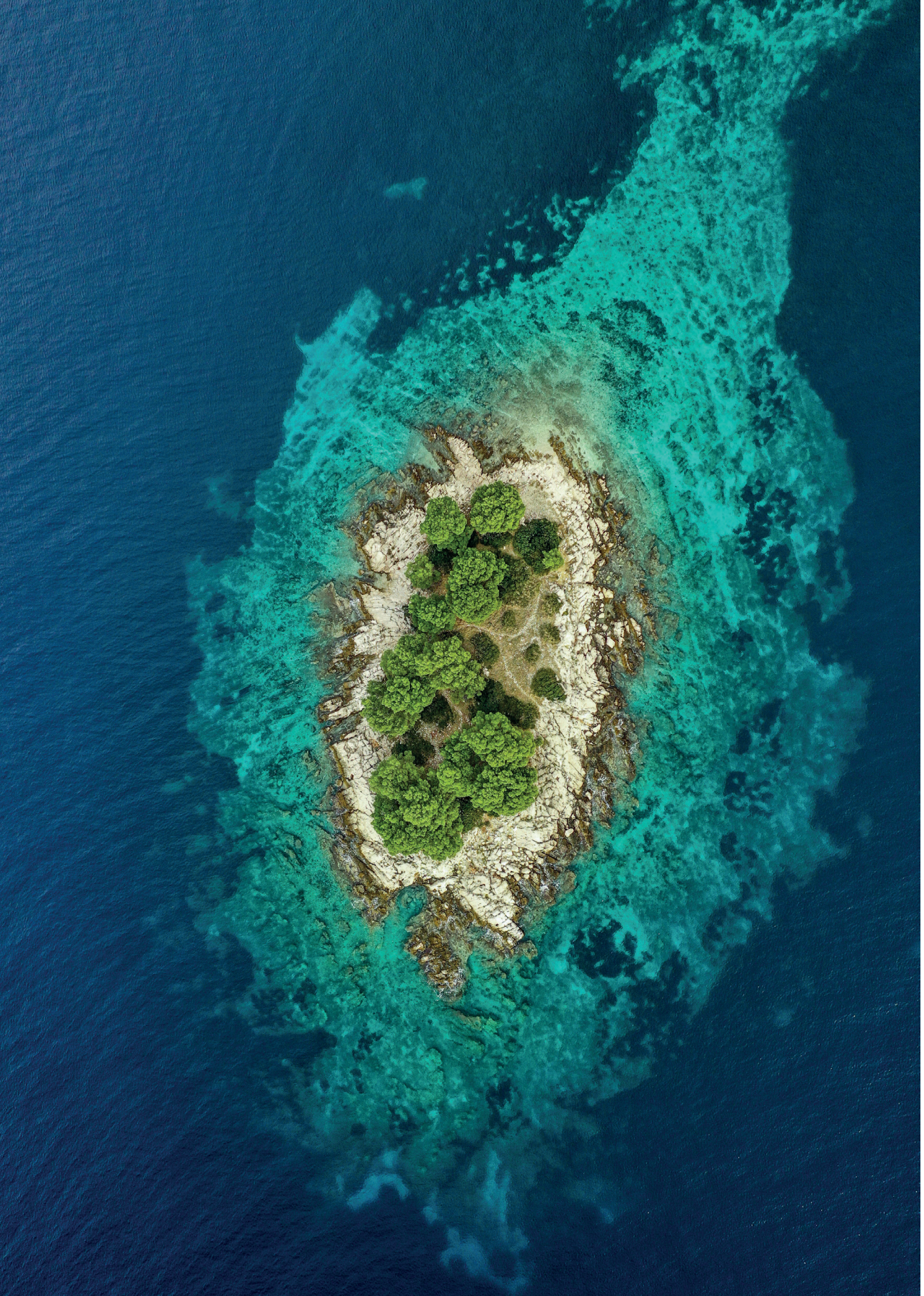
21. Waljee, A., Waljee, J., Morris, A. M. & Higgins, P. D. R. Threefold increased risk of infertility: a meta-analysis of infertility after ileal pouch anal anastomosis in ulcerative colitis. *Gut* **55,** 1575–80 (2006).

22. Hueting, W. E., Gooszen, H. G. & van Laarhoven, C. J. H. M. Sexual function and continence after ileo pouch anal anastomosis: a comparison between a meta-analysis and a questionnaire survey. *Int. J. Colorectal Dis.* **19,** 215–8 (2004).

23. Rajaratnam, S. G., Eglinton, T. W., Hider, P. & Fearnhead, N. S. Impact of ileal pouch-anal anastomosis on female fertility: meta-analysis and systematic review. *Int. J. Colorectal Dis.* **26,** 1365–74 (2011).

SECTION 2:

Improving detection and removal of premalignant lesions



CHAPTER 6

Surveillance of long-standing colitis: the role of image-enhanced endoscopy

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ABSTRACT

Patients with long-standing inflammatory bowel disease of the colon are at an increased risk of developing colorectal carcinoma. Surveillance programs have been implemented with the aim of detecting neoplastic lesions in an early stage. Due to limitations of conventional white light endoscopy, several new techniques to enhance the detection of dysplastic lesions in this setting have been explored. These advanced endoscopic techniques use a variety of methods to improve visualization, such as pancolonic dye-spraying (chromoendoscopy), optical filters (narrow-band imaging) and autofluorescence of mucosal tissue (autofluorescence imaging). At present, most guidelines have adopted chromoendoscopy as the preferred method for surveillance, based on several controlled studies. It is currently unknown if widespread implementation of chromoendoscopy will lead to an improved clinical outcome.

This review explores the current evidence on image-enhanced endoscopic techniques used in the detection of neoplastic lesions in patients with long standing colitis.

INTRODUCTION

Patients with inflammatory bowel disease (IBD) are at an increased risk of developing colorectal cancer (CRC).[1–3] This association has been shown for both ulcerative and Crohn's colitis[4] with cumulative risks estimated to amount to 18% for a disease duration of 30 years.[3] More recent population-based studies show a less distinct risk for the general IBD population[5–8], possibly owing to improved and more effective therapeutic regimens, the effects of surveillance or different kinds of bias in the early reports. Presently, the risk of CRC in the whole IBD population is estimated to be increased with a standardized incidence ratio of 1.7.[5] The highest risk is encountered in patients cared for in referral centers, patients with a longer disease duration and/or extensive colitis and in patients diagnosed before the age of 30, a concomitant diagnosis of primary sclerosing cholangitis (PSC) and a family history of CRC. [9–13] Recently, we created a prediction rule for IBD-associated CRC, based on the presence of PSC, extensive colitis and post-inflammatory polyps, which was found to reliably distinguish patients with a high or low risk of developing CRC.[14]

Endoscopic characterization of dysplastic lesions in colonic mucosa affected by IBD can be challenging, owing to the variable appearance of precancerous lesions. Mucosal abnormalities relating to colitis, both inflammatory and post-inflammatory (e.g. scarring or inflammatory polyps), may also cause difficulty in differentiating benign from dysplastic lesions. In addition, the occurrence of nonpolypoid dysplasia with minimal or no elevation, which is considered a frequently encountered phenotype in IBD[15], further hampers identification of dysplasia in this setting.

Until recently, endoscopically raised lesions containing dysplasia were termed dysplasia-associated lesions or masses (DALMs). Early studies reported high cancer rates in association with the occurrence of DALMs and therefore colectomy was advocated in these cases. [16,17] More recently, polyps with dysplasia found in an area of inflammation that have an endoscopic resemblance to sporadic adenomas (adenoma-like masses or ALMs) were described.[18,19] Several studies have shown that endoscopic resection of ALMs is feasible and safe if patients are closely followed-up thereafter.[19–21] Due to improvements in endoscopes, different classification of lesions

and changes in management decisions, several authors have suggested to abandon the DALM/ALM-nomenclature.[22,23] In addition, it is now widely acknowledged that nearly all dysplastic lesions are endoscopically visible.[24,25] Hence, for lesions in an area affected by inflammation, clinicians are increasingly pursuing a classification based on endoscopic resectability. Lesions that are both completely resectable and have negative biopsies from surrounding mucosa may indicate a better prognosis, in which case management can be based on endoscopic techniques rather than colectomy.[20,22]

Despite the ongoing debate on the true risk of IBD-associated neoplasia, there is a widely held consensus on the need for surveillance in patients with long-standing and extensive IBD of the colon. Most major guidelines state that surveillance should be initiated 8 to 10 years after diagnosis, or immediately when coexistent PSC is diagnosed. Depending on additional risk stratification, surveillance intervals may vary between 1 and 5 years.[26–31]

Until now, a direct link between early detection of dysplasia and a decrease in CRC-related mortality has not been evidently shown. A Cochrane systematic review was only able to include 3 studies that had a comparison group that had not received surveillance.[32] Although in these studies CRCs tended to be detected at an earlier stage, no clear effect on patient survival was shown. However, more recent retrospective studies have reported improved survival rates for patients undergoing surveillance.[33,34] Although a high percentage of interval CRCs, as observed in a British cohort followed for more than 30 years[7], might hamper the effectiveness of surveillance, a recent study on interval CRCs reported far lower incidence rates.[35]

Until recently, the established method for surveillance had been the combination of standard white light endoscopy and taking random biopsies. Traditionally, this involved taking 4-quadrant biopsies every 10 cm along the colon, aimed at retrieving a minimum of 33 biopsies.[36] This strategy has recently been found to be largely ineffective, with one estimated episode of dysplasia detected for every 1505 random biopsies taken.[37] This finding, and the notion that almost all IBD-associated dysplastic lesions are in fact endoscopically visible[24,25], prompted updates of several guidelines

in which the use of an image enhanced endoscopy technique (i.e. chromoendoscopy) was advocated.

In this review, we aim to provide an overview of the current evidence on the use of image-enhanced endoscopy (IEE) in detecting IBD-associated dysplasia and early-stage CRC.

CHROMOENDOSCOPY

Chromoendoscopy (CE) is a technique that involves the application of a dye to improve the delineation of mucosal abnormalities. (Figure 1) The most frequently studied staining solutions are indigo carmine (concentration 0.2%-0.4%), a contrast dye revealing the mucosal pit pattern, and methylene blue (concentration 0.1%), which acts as a contrast dye but is also absorbed better by normal mucosa than by areas of neoplasia and inflammation. During inspection the Kudo pit pattern is a valuable tool to classify the crypt architecture that becomes evident after dye spraying. Lesions classified as having a I-II pit pattern are generally considered to be of benign histology, while type III-V pit patterns tend to predict neoplastic lesions.[38]

Ten studies, of which 3 were parallel trials, aimed to compare CE and standard WLE for the detection of dysplastic lesions.[39–48] The first trial compared CE (using methylene blue 0.1% and magnification endoscopy) to standard definition WLE.[39] Patients were randomized into parallel groups and in both groups targeted as well as sequential biopsy specimens were taken. Significantly more intraepithelial neoplasia was found in the CE group compared to the WLE group (32 vs. 10, p=0.00315). The advantages of CE as reported in this landmark study were confirmed in most subsequent studies. (Table 1)

Subramanian et al published a meta-analysis in 2011, incorporating 1277 patients from 6 studies[39–44], and showed that the difference in diagnostic yield of any dysplasia was 7% (95% CI 3.2–11.3) in favour of CE over WLE. [49] This resulted in a number needed to treat of 14 patients to detect one additional patient with dysplasia. Pooling only the two studies using two separate (parallel) groups did not lead to a loss of significance for the increased yield. Although the evidence for the effectiveness of CE seems compelling, some limitations should be addressed. Some studies used a non-randomized crossover design.[44,46] Obviously, this design dictates a sequence in which white light endoscopy is always employed prior to the CE procedure. For that reason, the increased yield found in non-parallel CE trials may partially have resulted from the advantage of 'a second look' procedure. This phenomenon has been observed in WLE back-to-back studies in non-IBD individuals as well.[50] The impossibility of

blinding studies in which CE and WLE endoscopies are compared, and the fact that the use of dye implicates 2 introduction and 2 withdrawal episodes (as compared with a single introduction and withdrawal episode in WLE) might have resulted in additional bias as well.

The convincing evidence on the superiority of CE over standard definition WLE has prompted the major gastroenterological societies to adopt this technique as the new gold standard for neoplasia detection in IBD.

The SURFACE guidelines have been developed to aid the endoscopist in the application of CE in patients with IBD.[51] Importantly, these guidelines state that the CE technique should be avoided in patients with active disease or inadequate bowel preparation. In contrast, a large retrospective multicenter case-control study from the Netherlands in which dysplasia detection rates before and after implementation of CE were compared showed no benefit of CE over WLE.[52] This, and a more recent randomized controlled in which CE was not found to be superior to WLE[48] cast doubt on the assumed superiority of CE.

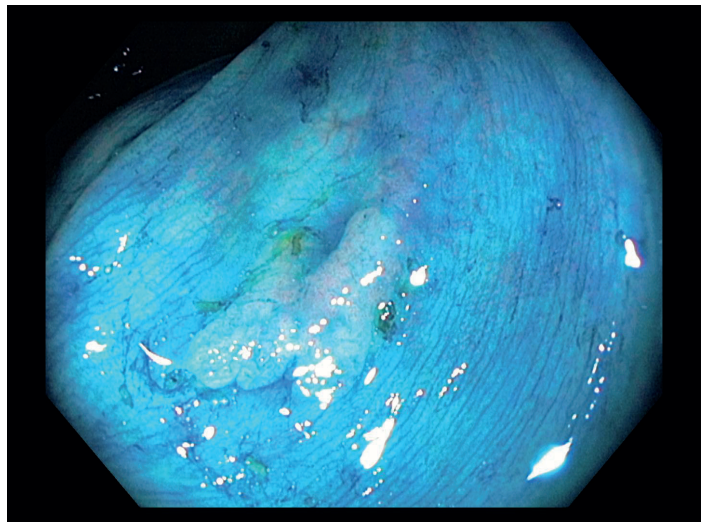


Figure 1. Mucosal lesion displayed using chromoendoscopy, after dye spraying with methylene blue. The lesion was subsequently removed using snare polypectomy and contained low-grade dysplasia.

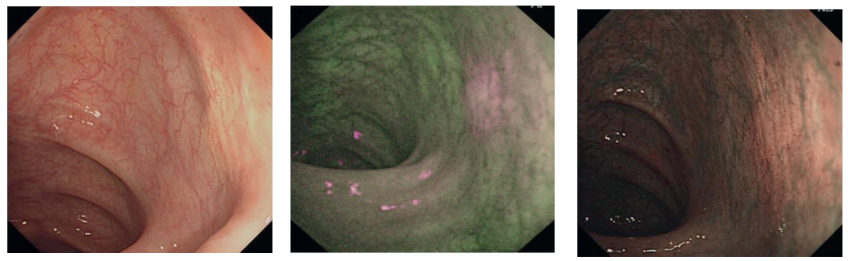


Figure 2. Mucosal lesion as imaged using (A) high-definition WLE, (B) AFI, in which the lesion is seen as a purple highlight, and (C) NBI, showing subtle enhancement. Biopsy of the lesion revealed low-grade dysplasia.
Used with permission from: Evelien Dekker, MD PhD, Department of Gastroenterology and Hepatology, Academic Medical Centre, Amsterdam, the Netherlands

Virtual imaging techniques (NBI, FICE, i-scan)

In addition to CE, other IEE techniques have been developed in an attempt to improve the endoscopic diagnostic accuracy. These techniques include narrow-band imaging (NBI) and virtual techniques like Fujifilm Intelligent Color Enhancement (FICE) and i-scan. Optical IEE without dye-spraying can be performed using compound-band

imaging (CBI) or the more frequently studied narrow-band imaging (NBI) (Olympus, Tokyo, Japan). Both FICE and i-scan use a digital technique, in which the image enhancement occurs later by using specialized software.

Narrow-band imaging

The optical technique used in NBI is based on narrowing the spectrum of light emitted from the endoscope. Depth of penetration of light into the mucosa is dependent on its wavelength (i.e. deep for red light and superficial for blue light) while the spectrum of reflected light depends on tissue composition, for example inflammation or dysplasia. The filters used in NBI narrow the projected light down into blue (415 nm) and green (540 nm) wavelength bands, which leads to better visualization of superficial vasculature and mucosal patterns.

Studies comparing NBI to WLE

The first study comparing NBI to standard definition WLE in the detection of neoplastic lesions in IBD surveillance had a randomized crossover design and made use of first generation NBI equipment.[53] In this study the number of visible lesions was higher for NBI, but this came at a cost: the number of false positive lesions was increased as well (43 vs. 16, p=0.015). There was no significant difference in the number of histologically proven neoplastic lesions between the two groups. A disadvantage of the first generation of NBI equipment was darkening of the visual field, but this has been overcome with the development of a newer generation that offers improved light intensity. Crossover trials published later on also failed to show a benefit of NBI in the detection of true-positive lesions, though.[54] In 2012, Ignjatovic et al published the results of a parallel-arm randomized controlled trial comparing second generation NBI to high-definition WLE.[55] Similar to other trials, no significant difference in the detection of dysplasia in targeted biopsies was found. The authors also took random biopsies in both study arms, which revealed only one dysplastic lesion in 2,707 samples (dysplasia yield 0.04%). The results of these studies indicate that NBI is not likely to improve the detection rate of dysplasia in IBD surveillance and

underscore the futility of random mucosal sampling. Still, NBI might prove valuable as an adjunct after lesion detection by increasing the overall specificity of surveillance. [56]

Studies comparing NBI to CE

Two published crossover trials have directly compared NBI to CE. Pellisé et al performed a trial in which 60 patients were randomly assigned to the order of the two procedures. [57] The number of false-positives was significantly less for NBI (126 lesions vs. 196 lesions, p=0.001), but the number of true-positive lesions was not significantly different (NBI: 10 lesions vs. CE: 12 lesions, p=0.644). The additional lesions found on the second pass endoscopy resulted in miss rates of 31.8% for NBI and 13.6% for CE, which was not significantly different. Equivalence in dysplasia detection rates between NBI and CE was later also shown by Bisschops et al.[58]

A recent trial with a back-to-back design was published in 2013.[59] The number of suspected lesions was higher in the CE-group compared to the NBI-group (131 lesions vs. 102 lesions, p<0.001). Most of these lesions showed no dysplasia, but the difference in the number of neoplastic lesions showed a trend in favour of CE. The total miss rate, however, was 14% (3 dysplastic lesions) higher in NBI, which is in line with data from the Pellise study.[57] It has been suggested that the inferior sensitivity of NBI can be attributed to the fact that vascular patterns are more subtle than crypt architecture in the setting of colitis. Furthermore, despite the development of newer generation NBI systems, inferior light intensity may still be an issue. Considering that in the setting of a screening program the sensitivity should be of higher concern than specificity, current consensus is that NBI should not replace CE.

Table 1. Prospective studies investigating image enhanced endoscopic techniques in colitis surveillance. CE = chromoendoscopy, CLE = confocal laser endomicroscopy, NBI = narrow-band imaging, AFI = autofluorescence imaging, MB = methylene blue, IC = indigo carmine, SD = standard definition, HD = high definition, S = significant, NS = non-significant.

	Author	Year	N	Study Design	Intervention	Control	Dysplastic lesions	Significance
CE	Kiesslich [41]	2003	165	Parallel randomized	CE (MB 0.1%)	WLE (SD)	CE 32 (84 pts) vs WLE 10 (81 pts)	S
	Rutter [43]	2004	100	Back-to-back (WLE first)	CE (IC 0.1%)	WLE (SD)	CE 9 vs WLE 2	NS
	Kiesslich [45]	2007	153	Parallel randomized	CE (MB 0.1%) + CLE	WLE (SD)	CE 19 (80 pts) vs WLE 4 (73 pts)	S
	Marion [46]	2008	102	Back-to-back (WLE first)	CE (MB 0.1%)	WLE (SD)	CE 17 vs WLE 3	S
	Hlavaty [48]	2011	20	Back-to-back (WLE first)	CE (IC 0.4%) + CLE	WLE (SD)	CE 7 vs WLE 0	S
	Picco [49]	2013	75	Back-to-back (WLE first)	CE (IC 0.2%)	WLE (SD)	CE 22 vs WLE 10	S
	Freire [50]	2014	145	Parallel randomized	CE (MB 0.1%) + CLE	WLE (SD)	CE 7 (72 pts) vs. WLE 6 (73 pts)	NS
NBI	Dekker [55]	2007	42	Back-to-back (randomized)	NBI (first generation)	WLE (SD)	NBI 9 vs WLE 12	NS
	Van den Broek [56]	2011	48	Back-to-back (randomized)	NBI	WLE (HD)	NBI 13 vs WLE 11	NS
	Pellise [59]	2011	60	Back-to-back (randomized)	NBI	CE	NBI 10 vs CE 12	NS
	Ignjatovic [57]	2012	112	Parallel randomized	NBI	WLE (HD)	NBI 5 (56 pts) vs WLE 7 (56 pts)	NS
	Efthymiou [61]	2013	144	Back-to-back (NBI first)	NBI	CE	NBI 20 vs WLE 23	NS
AFI	van den Broek [63]	2008	25	Back-to-back (randomized)	AFI	WLE (HD)	AFI 16 vs WLE 13	S

FICE and i-scan

Digital chromoendoscopy (digital DLC) is carried out using either FICE (Fujifilm, Tokyo, Japan) or i-scan (Pentax, Tokyo, Japan). The endoscopic image is constructed digitally in real time by enhancing the intensity of reflected narrowed blue light and reduce reflected red and green light. In this way, a similar image to NBI is constructed, but instead of altering the spectrum of emitted light, FICE and i-scan make digital adjustments. These two methods have not been studied in the context IBD surveillance as of yet.

Autofluorescence imaging

Autofluorescence imaging (AFI) is a fairly new imaging technique used to create a color contrast between normal mucosa and neoplastic tissue. The mechanism used is based on the property of fluorophores to emit autofluorescence when exposed to ultraviolet light. This fluorescent light, which has a longer wavelength, creates purple highlights of abnormal tissue on a green background of normal mucosa. (Figure 2)

A meta-analysis assessing the ability of several IEE-techniques to differentiate sporadic colonic polyps found an overall sensitivity of 86.7% for AFI.[60] However, data on AFI used specifically in surveillance of IBD patients are sparse. To date, only one trial comparing AFI with WLE has been published.[61] All fifty patients in this trial underwent surveillance colonoscopy with both AFI and WLE in random order. In the group that underwent WLE first, a miss rate of 50% was observed (3 out of 6 additional dysplastic lesions were found using AFI), while the AFI-first group had a miss rate of 0% (no additional lesions were detected during the successive WLE procedure). Moreover, the increased yield with AFI did not lead to a significant increase in the rate of false positive biopsies.

Although no comparative trials on the use of AFI in IBD surveillance have been published since, Matsumoto et al published a prospective study using AFI as an adjunct to WLE.[56] In 48 patients, all lesions identified by WLE were detected again by using AFI and further classified as having either normal (green) or low autofluorescence (purple). The rate of dysplasia in 126 lesions was not significantly

different for the two AFI classifications (14% for low AF vs. 5% for normal AF, p=0.09). However, the study was not designed to assess the effectiveness of initial dysplasia detection.

Confocal laser endomicroscopy

Confocal laser endomicroscopy (CLE) is a magnification technique that assists the endoscopist in differentiation of lesions, once detected. CLE works by filtering the returned light from a low-power laser aimed at the mucosal surface, creating a real-time microscopic image.[62]

Several studies have used CLE as an add-on to CE, in an attempt to improve the specificity of a surveillance colonoscopy.[43,45,46,48] Kiesslich et al demonstrated a high diagnostic accuracy with a sensitivity of 94.7% and specificity of 98.3% for the detection of neoplasia in a total of 137 lesions.[43] In a smaller series, CLE had a high diagnostic accuracy for 14 suspicious lesions found during CE (positive predictive value 83%, negative predictive value 100%).[63] In this way, CLE seems a valuable tool in increasing the per-biopsy yield, yet it is not suitable as a flagging technique, i.e. for the detection of lesions during surveillance. Its implementation is also held back by high equipment costs and the fact that it requires the endoscopist to make a histological diagnosis.

Interestingly, CLE may also be used in the assessment of inflammatory activity on a microscopic level during endoscopy. Several reports have shown its usefulness in grading disease activity, which could aid the clinician in predicting disease course and choosing the best management for the individual patient.[64,65]

Endocytoscopy

Endocytoscopy is a technique that provides high (up to 1400 fold) magnification in vivo, utilizing a contact light microscope.[66] After application of a topical contrast agent, the cellular structure of the superficial mucosa can be visualized and differentiated.[67–69] Although this evolving technique has not yet been studied in

IBD surveillance, the obvious clinical positioning includes differentiation of lesions suspect for neoplasia. Much the same as CLE, this procedure can only serve as an adjunct to other endoscopic techniques, requires extensive training and is considered time-consuming.

Table 2. Summary of characteristics for each advanced endoscopic technique. HD = high definition, CE = chromoendoscopy, NBI = narrow-band imaging, CLE = confocal laser endomicroscopy, AFI = autofluorescence imaging.

	Evidence for accuracy	Number of published trials	Guideline recommendation	Ease of use	Additional training required
Detection techniques					
HD-WLE	+/-	2 (vs NBI)	+ (if CE not available)	+	-
CE	+	7	+	+/-	+/-
NBI	-	5	-	+	+/-
FICE/i-scan	-	0	-	+	+
Adjuvant techniques					
AFI	+	1	-	+/-	+
CLE/Endocytoscopy	-	-	-	-	+

Implementation

According to the available evidence, the increased risk of colorectal cancer in a subset of patients with long-standing colitis warrants enrolment of these patients in surveillance programs. Discussions on implementation of new screening programs should be guided by valid and relevant criteria.[70,71] Important issues that have not been fully settled are the use of risk stratification-based screenings algorithms, the effectiveness of the available techniques and the need for training when novel methods are introduced. Before the clinical introduction of new techniques, their superiority over existing methods should be convincingly established. First, the detection rate of dysplastic lesions should be at least as high as the rates reported employing current gold standard. Second, new techniques ought to offer better

differentiation of lesions, once detected. Third, the number of redundant biopsies should be reduced to a minimum, without reducing the sensitivity for dysplasia detection.

From a practical point of view, several additional principles should be considered. For instance, the economic costs of implementation should be proportional to the added gains. Furthermore, new techniques should not result in an increased procedure time and must be safe and acceptable to the patient.

Considering this, random mucosal sampling has largely been considered obsolete, because it leads to an imbalanced expenditure of resources[37,47], though some guidelines still recommend it as a 'back up' procedure. To date, the most compelling evidence for improved dysplasia detection is still in favour of CE, although this technique has several limitations such as the pooling of dye and the inability to properly inspect the capillary network. For NBI the main drawback has been a low image brightness, in particular when using first generation devices. Practical disadvantages to using AFI appear to be a low image resolution and the possible interruption of autofluorescence in case of insufficient bowel preparation. In general, dye-less techniques such as NBI are considered easier in use, since the advanced imaging is established by a flick of a switch rather than through pancolonic dye spraying.

The endoscopic techniques presented in this review require additional training in varying intensity. While some techniques are considered more difficult than others, comparative data on the different learning curves are not available. For CE, the practice of dye spraying is considered to be straightforward, but the visual interpretation of lesions depends on training. Picco et al recently investigated interobserver agreement and withdrawal time for CE when performed by an inexperienced endoscopist.[72] The interobserver agreement between six endoscopists was high, with kappa-scores of 0.91 for WLE and 0.86 for CE.

Dai et al studied the effectiveness of a limited training programme for the interpretation of NBI images in patients with colorectal lesions and found excellent

overall sensitivity and specificity after the first set of training images (98.0% and 92.0% respectively).[73]

CE is considered to be more time-consuming than other IEE techniques, owing to the dye-spraying prior to inspection. Subramanian et al calculated a pooled weighted difference of 11 minutes in duration between the CE and WLE procedures.[49] In contrast, the virtual techniques are generally believed to have shorter withdrawal times than CE. Pellise et al found a significantly reduced mean withdrawal time for NBI compared to CE (15.74 vs. 26.87 minutes, p=0.01).[57] In contrast, Efthymiou et al found similar examination times for NBI and CE (13 vs. 13 minutes).[59]

It is crucial that costs are taken into account as well. The acquisition and use of modern instrumentation are costly, yet may ultimately lead to decreased pathology-related expenditure. Unfortunately, data on the cost-effectiveness of the IEE-techniques discussed here is sparse and hard to interpret. Konijeti et al performed a cost-effectiveness analysis comparing three strategies for surveillance (CE, WLE with random biopsies and no surveillance).[74] The investigators modeled the risk of CRC on data from a meta-analysis by Jess et al (4% cumulative risk at 25 years of disease).[6] CE was more cost-effective than WLE at all surveillance intervals. However, in the model the investigators used, CE became only cost-effective at surveillance intervals of 7 years, which is significantly longer than currently advised interval durations. It is as of yet unclear if the introduction of IEE techniques may also grant a decrease in the number of colonoscopies over time.

Over time, the effectiveness of conventional colonoscopy has also improved considerably with the advent of state-of-the-art, high-definition equipment (HD-WLE).[75 76] However, evidence on the potential future role of HD-WLE in IBD surveillance is limited. A large part of the studies cited in guidelines that now advocate CE used standard definition WLE in their control groups. Additional comparative trials should clarify whether these claims also hold true if HD-WLE is used as comparator.

REFERENCES

1. Ekbom, A., Helmick, C., Zack, M. & Adami, H. O. Ulcerative colitis and colorectal cancer. A population-based study. *N. Engl. J. Med.* **323,** 1228–33 (1990).
2. Bernstein, C. N., Blanchard, J. F., Kliewer, E. & Wajda, A. Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* **91,** 854–62 (2001).
3. Eaden, J. A., Abrams, K. R. & Mayberry, J. F. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* **48,** 526–35 (2001).
4. Gillen, C. D., Walmsley, R. S., Prior, P., Andrews, H. A. & Allan, R. N. Ulcerative colitis and Crohn's disease: a comparison of the colorectal cancer risk in extensive colitis. *Gut* **35,** 1590–2 (1994).
5. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
6. Jess, T. *et al.* Decreasing risk of colorectal cancer in patients with inflammatory bowel disease over 30 years. *Gastroenterology* **143,** 375-81–4 (2012).
7. Rutter, M. D. *et al.* Thirty-year analysis of a colonoscopic surveillance program for neoplasia in ulcerative colitis. *Gastroenterology* **130,** 1030–8 (2006).
8. Herrinton, L. J. *et al.* Incidence and mortality of colorectal adenocarcinoma in persons with inflammatory bowel disease from 1998 to 2010. *Gastroenterology* **143,** 382–9 (2012).
9. Levin, B. Inflammatory bowel disease and colon cancer. *Cancer* **70,** 1313–6 (1992).
10. Söderlund, S. *et al.* Decreasing time-trends of colorectal cancer in a large cohort of patients with inflammatory bowel disease. *Gastroenterology* **136,** 1561-7-9 (2009).
11. Soetikno, R. M., Lin, O. S., Heidenreich, P. A., Young, H. S. & Blackstone, M. O. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. *Gastrointest. Endosc.* **56,** 48–54 (2002).
12. Askling, J. *et al.* Colorectal cancer rates among first-degree relatives of patients with inflammatory bowel disease: a population-based cohort study. *Lancet* **357,** 262–6 (2001).
13. Loftus, E. V. Epidemiology and risk factors for colorectal dysplasia and cancer in ulcerative colitis. *Gastroenterol. Clin. North Am.* **35,** 517–31 (2006).
14. Lutgens, M. *et al.* A rule for determining risk of colorectal cancer in patients with inflammatory bowel disease. *Clin. Gastroenterol. Hepatol.* **13,** 148–54.e1 (2015).
15. Jaramillo, E. *et al.* Small, flat colorectal neoplasias in long-standing ulcerative colitis detected by high-resolution electronic video endoscopy. *Gastrointest. Endosc.* **44,** 15–22 (1996).

16. Blackstone, M. O., Riddell, R. H., Rogers, B. H. & Levin, B. Dysplasia-associated lesion or mass (DALM) detected by colonoscopy in long-standing ulcerative colitis: an indication for colectomy. *Gastroenterology* **80,** 366–74 (1981).

17. Odze, R. D. Adenomas and adenoma-like DALMs in chronic ulcerative colitis: a clinical, pathological, and molecular review. *Am. J. Gastroenterol.* **94,** 1746–50 (1999).

18. Neumann, H., Vieth, M., Langner, C., Neurath, M. F. & Mudter, J. Cancer risk in IBD: How to diagnose and how to manage DALM and ALM. *World Journal of Gastroenterology* **17,** 3184–3191 (2011).

19. Wanders, L. K. *et al.* Cancer risk after resection of polypoid dysplasia in patients with longstanding ulcerative colitis: a meta-analysis. *Clin. Gastroenterol. Hepatol.* **12,** 756–64 (2014).

20. Odze, R. D., Farraye, F. A., Hecht, J. L. & Hornick, J. L. Long-term follow-up after polypectomy treatment for adenoma-like dysplastic lesions in ulcerative colitis. *Clin. Gastroenterol. Hepatol.* **2,** 534–41 (2004).

21. Rubin, P. H. *et al.* Colonoscopic polypectomy in chronic colitis: conservative management after endoscopic resection of dysplastic polyps. *Gastroenterology* **117,** 1295–300 (1999).

22. Allen, P. B., Kamm, M. A., De Cruz, P. & Desmond, P. V. Dysplastic lesions in ulcerative colitis: changing paradigms. *Inflamm. Bowel Dis.* **16,** 1978–83 (2010).

23. Rutter, M. D. Importance of nonpolypoid (flat and depressed) colorectal neoplasms in screening for CRC in patients with IBD. *Gastrointest. Endosc. Clin. N. Am.* **24,** 327–35 (2014).

24. Rutter, M. D. *et al.* Most dysplasia in ulcerative colitis is visible at colonoscopy. *Gastrointest. Endosc.* **60,** 334–9 (2004).

25. Rubin, D. T., Rothe, J. A., Hetzel, J. T., Cohen, R. D. & Hanauer, S. B. Are dysplasia and colorectal cancer endoscopically visible in patients with ulcerative colitis? *Gastrointest. Endosc.* **65,** 998–1004 (2007).

26. Farraye, F. A. *et al.* AGA medical position statement on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* **138,** 738–45 (2010).

27. Laine, L. *et al.* SCENIC International Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease. *Gastroenterology* **148,** 639–651.e28 (2015).

28. Cairns, S. R. *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* **59,** 666–689 (2010).

29. Annese, V. *et al.* European evidence based consensus for endoscopy in inflammatory bowel disease. *J. Crohns. Colitis* **7,** 982–1018 (2013).

30. Kamiński, M. *et al.* Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* **46,** 435–457 (2014).

31. London: National Institute for Health and Clinical Excellence (UK); 2011 Mar. Colonoscopic Surveillance for Prevention of Colorectal Cancer in People with Ulcerative Colitis, Crohn's Disease or Adenomas - PubMed - NCBI. Available at: https://www.ncbi.nlm.nih.gov/d/?term=Centre+for+Clinical+Practice+at+NICE+(UK).+Colonoscopic+Surveillance+for+Prevention+of. (Accessed: 23rd July 2019)

32. Collins, P. D., Mpofu, C., Watson, A. J. & Rhodes, J. M. Strategies for detecting colon cancer and/or dysplasia in patients with inflammatory bowel disease. *Cochrane database Syst. Rev.* CD000279 (2006). doi:10.1002/14651858.CD000279.pub3

33. Lutgens, M. W. M. D. *et al.* Colonoscopic surveillance improves survival after colorectal cancer diagnosis in inflammatory bowel disease. *Br. J. Cancer* **101,** 1671–5 (2009).

34. Boonstra, K. *et al.* Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. *Hepatology* **58,** 2045–2055 (2013).

35. Mooiweer, E. *et al.* Incidence of Interval Colorectal Cancer Among Inflammatory Bowel Disease Patients Undergoing Regular Colonoscopic Surveillance. *Clin. Gastroenterol. Hepatol.* **13,** 1656–1661 (2015).

36. Itzkowitz, S. H., Present, D. H. & Crohn's and Colitis Foundation of America Colon Cancer in IBD Study Group. Consensus conference: Colorectal cancer screening and surveillance in inflammatory bowel disease. *Inflamm. Bowel Dis.* **11,** 314–21 (2005).

37. Rutter, M. D. & Riddell, R. H. Colorectal dysplasia in inflammatory bowel disease: a clinicopathologic perspective. *Clin. Gastroenterol. Hepatol.* **12,** 359–67 (2014).

38. Kudo, S. *et al.* Diagnosis of colorectal tumorous lesions by magnifying endoscopy. *Gastrointest. Endosc.* **44,** 8–14 (1996).

39. Kiesslich, R. *et al.* Methylene blue-aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology* **124,** 880–8 (2003).

40. Matsumoto, T., Nakamura, S., Jo, Y., Yao, T. & Iida, M. Chromoscopy might improve diagnostic accuracy in cancer surveillance for ulcerative colitis. *Am. J. Gastroenterol.* **98,** 1827–33 (2003).

41. Rutter, M. D. Pancolonic indigo carmine dye spraying for the detection of dysplasia in ulcerative colitis. *Gut* **53,** 256–260 (2004).

42. Hurlstone, D., Sanders, D., Lobo, A., McAlindon, M. & Cross, S. Indigo Carmine-Assisted High-Magnification Chromoscopic Colonoscopy for the Detection and Characterisation of Intraepithelial Neoplasia in Ulcerative Colitis: A Prospective Evaluation. *Endoscopy* **37,** 1186–1192 (2005).

43. Kiesslich, R. *et al.* Chromoscopy-guided endomicroscopy increases the diagnostic yield of intraepithelial neoplasia in ulcerative colitis. *Gastroenterology* **132,** 874–82 (2007).

44. Marion, J. F. *et al.* Chromoendoscopy-targeted biopsies are superior to standard colonoscopic surveillance for detecting dysplasia in inflammatory bowel disease patients: a prospective endoscopic trial. *Am. J. Gastroenterol.* **103,** 2342–9 (2008).

45. Günther, U. *et al.* Surveillance colonoscopy in patients with inflammatory bowel disease: comparison of random biopsy vs. targeted biopsy protocols. *Int. J. Colorectal Dis.* **26,** 667–72 (2011).

46. Hlavaty, T. *et al.* Colorectal cancer screening in patients with ulcerative and Crohn's colitis with use of colonoscopy, chromoendoscopy and confocal endomicroscopy. *Eur. J. Gastroenterol. Hepatol.* **23,** 680–9 (2011).

47. Picco, M. F. *et al.* Procedure time and the determination of polypoid abnormalities with experience: implementation of a chromoendoscopy program for surveillance colonoscopy for ulcerative colitis. *Inflamm. Bowel Dis.* **19,** 1913–20 (2013).

48. Freire, P. *et al.* Surveillance in ulcerative colitis: is chromoendoscopy-guided endomicroscopy always better than conventional colonoscopy? A randomized trial. *Inflamm. Bowel Dis.* **20,** 2038–45 (2014).

49. Subramanian, V., Mannath, J., Ragunath, K. & Hawkey, C. J. Meta-analysis: the diagnostic yield of chromoendoscopy for detecting dysplasia in patients with colonic inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **33,** 304–12 (2011).

50. Rex, D. *et al.* Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology* **112,** 24–28 (1997).

51. Kiesslich, R. & Neurath, M. F. Chromoendoscopy: an evolving standard in surveillance for ulcerative colitis. *Inflamm. Bowel Dis.* **10,** 695–6 (2004).

52. Mooiweer, E. *et al.* Chromoendoscopy for Surveillance in Inflammatory Bowel Disease Does Not Increase Neoplasia Detection Compared With Conventional Colonoscopy With Random Biopsies: Results From a Large Retrospective Study. *Am. J. Gastroenterol.* **110,** 1014–21 (2015).

53. Dekker, E. *et al.* Narrow-band imaging compared with conventional colonoscopy for the detection of dysplasia in patients with longstanding ulcerative colitis. *Endoscopy* **39,** 216–221 (2007).

54. van den Broek, F. *et al.* Narrow-band imaging versus high-definition endoscopy for the diagnosis of neoplasia in ulcerative colitis. *Endoscopy* **43,** 108–115 (2011).

55. Ignjatovic, A. *et al.* Narrow Band Imaging for Detection of Dysplasia in Colitis: A Randomized Controlled Trial. *Am. J. Gastroenterol.* **107,** 885–890 (2012).

56. Matsumoto, T. *et al.* Magnifying colonoscopy with narrow band imaging system for the diagnosis of dysplasia in ulcerative colitis: a pilot study. *Gastrointest. Endosc.* **66,** 957–65 (2007).

57. Pellisé, M. *et al.* Narrow-band imaging as an alternative to chromoendoscopy for the detection of dysplasia in long-standing inflammatory bowel disease: a prospective, randomized, crossover study. *Gastrointest. Endosc.* **74,** 840–848 (2011).

58. Baert FJ, et al. B. R. B. T. Chromo-endoscopy versus narrow band imaging in ulcerative colitis: a prospective randomized controlled trial. *Gastrointest Endosc* **44,** (2012).

59. Efthymiou, M. *et al.* Chromoendoscopy versus narrow band imaging for colonic surveillance in inflammatory bowel disease. *Inflamm. Bowel Dis.* **19,** 2132–8 (2013).

60. Wanders, L. K., East, J. E., Uitentuis, S. E., Leeflang, M. M. G. & Dekker, E. Diagnostic performance of narrowed spectrum endoscopy, autofluorescence imaging, and confocal laser endomicroscopy for optical diagnosis of colonic polyps: a meta-analysis. *Lancet Oncol.* **14,** 1337–1347 (2013).

61. van den Broek, F. J. C. *et al.* Endoscopic tri-modal imaging for surveillance in ulcerative colitis: randomised comparison of high-resolution endoscopy and autofluorescence imaging for neoplasia detection; and evaluation of narrow-band imaging for classification of lesions. *Gut* **57,** 1083–9 (2008).

62. Wallace, M. B. & Fockens, P. Probe-based confocal laser endomicroscopy. *Gastroenterology* **136,** 1509–13 (2009).

63. Rispo, A. *et al.* Diagnostic accuracy of confocal laser endomicroscopy in diagnosing dysplasia in patients affected by long-standing ulcerative colitis. *World J. Gastrointest. Endosc.* **4,** 414–20 (2012).

64. Neumann, H. *et al.* Assessment of Crohn´s disease activity by confocal laser endomicroscopy. *Inflamm. Bowel Dis.* **18,** 2261–2269 (2012).

65. Kiesslich, R. *et al.* Local barrier dysfunction identified by confocal laser endomicroscopy predicts relapse in inflammatory bowel disease. *Gut* **61,** 1146–53 (2012).

66. Kwon, R. S. *et al.* Endocytoscopy. *Gastrointest. Endosc.* **70,** 610–613 (2009).

67. Pohl, H. *et al.* Endocytoscopy for the detection of microstructural features in adult patients with celiac sprue: a prospective, blinded endocytoscopy-conventional histology correlation study. *Gastrointest. Endosc.* **70,** 933–41 (2009).

68. Neumann, H., Vieth, M. & Neurath, M. F. Image of the month. Endocytoscopy-based detection of focal high-grade intraepithelial neoplasia in colonic polyps. *Clin. Gastroenterol. Hepatol.* **9,** e13 (2011).

69. Kudo, S.-E. *et al.* Diagnosis of colorectal lesions with a novel endocytoscopic classification – a pilot study. *Endoscopy* **43,** 869–875 (2011).

70. Wilson JMG, J. G. Principles and practice of screening for disease. *Geneva WHO* (1968).

71. van den Broek, F. J. C. *et al.* Random biopsies taken during colonoscopic surveillance of patients with longstanding ulcerative colitis: low yield and absence of clinical consequences. *Am. J. Gastroenterol.* **109,** 715–22 (2014).

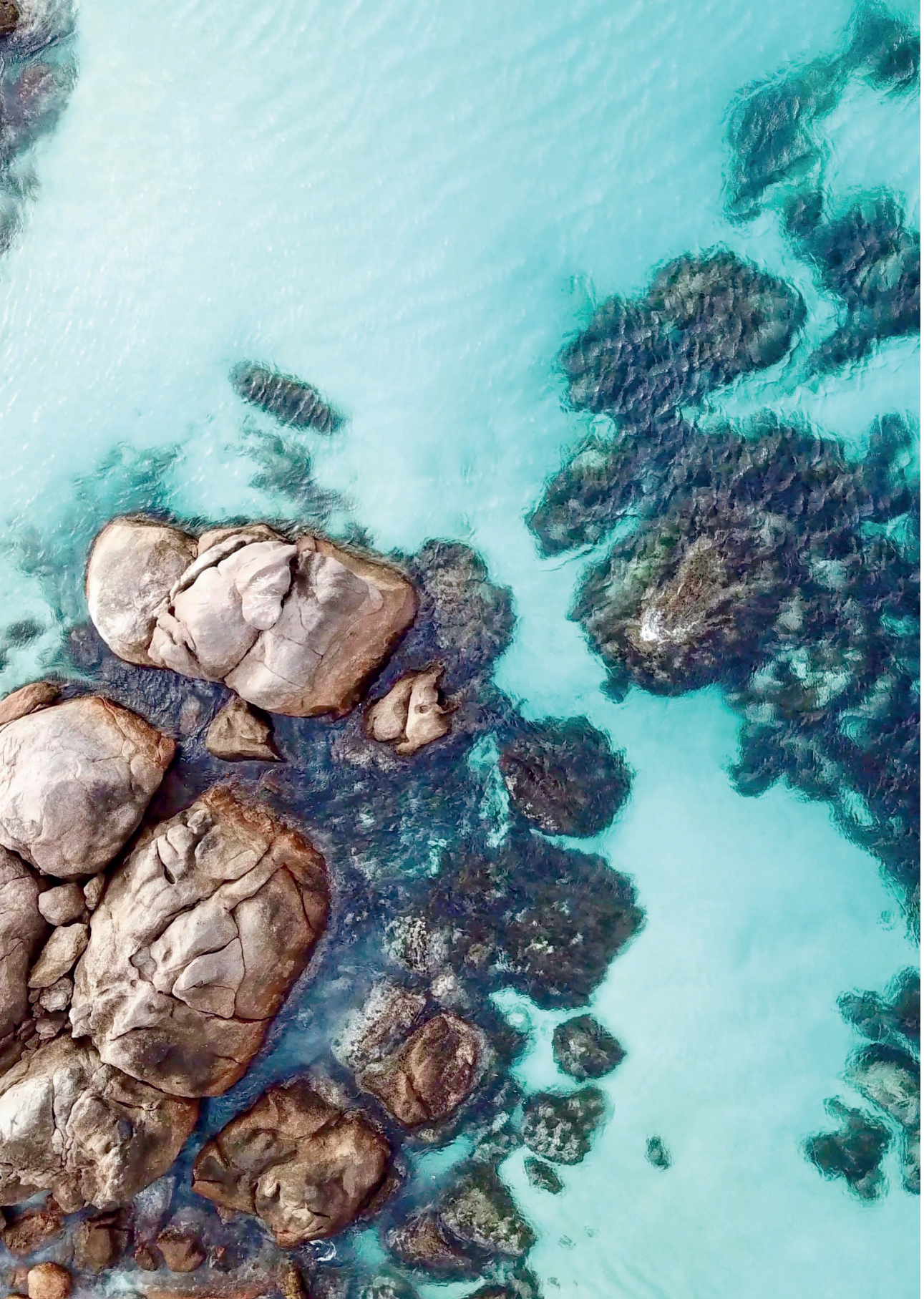
72. Dai, J. *et al.* Evaluation of narrow-band imaging in the diagnosis of colorectal lesions: is a learning curve involved? *Dig. Endosc.* **25,** 180–8 (2013).

73. Konijeti, G. G., Shrime, M. G., Ananthakrishnan, A. N. & Chan, A. T. Cost-effectiveness analysis of chromoendoscopy for colorectal cancer surveillance in patients with ulcerative colitis. *Gastrointest. Endosc.* **79,** 455–65 (2014).

74. Buchner, A. M. *et al.* High-definition colonoscopy detects colorectal polyps at a higher rate than standard white-light colonoscopy. *Clin. Gastroenterol. Hepatol.* **8,** 364–70 (2010).

75. Subramanian, V., Mannath, J., Hawkey, C. J. & Ragunath, K. High definition colonoscopy vs. standard video endoscopy for the detection of colonic polyps: a meta-analysis. *Endoscopy* **43,** 499–505 (2011).

76. Subramanian, V. *et al.* Comparison of high definition with standard white light endoscopy for detection of dysplastic lesions during surveillance colonoscopy in patients with colonic inflammatory bowel disease. *Inflamm. Bowel Dis.* **19,** 350–5 (2013).



CHAPTER 7

Low Rate of Dysplasia Detection in Mucosa Surrounding Dysplastic Lesions in Patients Undergoing Surveillance for Inflammatory Bowel Diseases

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ABSTRACT

Background & Aims: When dysplastic lesions are encountered during surveillance colonoscopy of patients with inflammatory bowel diseases (IBD), guidelines recommend collection of additional biopsies from the surrounding mucosa, to ensure the lesion has been adequately circumscribed. We aimed to determine the rate of dysplasia in mucosa biopsies collected from tissues surrounding dysplastic lesions during IBD surveillance.

Methods: In a retrospective study, we collected endoscopy and pathology reports from 1065 patients undergoing colonoscopic surveillance for IBD, from 2000 through 2015, at 3 centers in the Netherlands. We analyzed reports from all patients with dysplastic lesions from whom biopsies of surrounding mucosa were collected. Among 194 patients with 1 or more visible dysplastic lesion, mucosal biopsies were collected from tissues adjacent to 140 dysplastic lesions, from 71 patients (63% male; 48% with ulcerative colitis, 42% with Crohn's disease, and 10% with indeterminate colitis).

Results: The mean number of surrounding mucosa biopsies collected per lesion was 3.4 (range 1–6). Dysplasia was detected in 7 biopsies surrounding 140 areas of dysplasia (5.0%) and 5 biopsies surrounding 136 areas of low-grade dysplasia (3.7%). Dysplasia in biopsies of surrounding mucosa could be observed during 5/87 white-light endoscopies and during 2/53 chromoendoscopies. In patients with dysplasia in mucosa surrounding lesions of low-grade dysplasia, post-resection surveillance did not reveal high-grade dysplasia or colorectal cancer.

Conclusion: Dysplasia is detected in only 5% of biopsies collected from mucosa surrounding dysplastic lesions. This observation indicates that endoscopists accurately delineate the borders of dysplastic lesions during surveillance of patients with IBD. The lack of clinical consequences from routinely collecting biopsies from areas surrounding dysplastic lesions casts doubt on the usefulness and cost-effectiveness of this practice.

INTRODUCTION

Patients with inflammatory bowel disease (IBD) have an increased risk of colorectal cancer (CRC).[1,2] Surveillance colonoscopies aimed at the detection and removal of dysplastic lesions, thereby preventing cancer and CRC-related mortality, are recommended to reduce this risk.[3] Some guidelines still advise to take 4-quadrant random biopsies, every 10 cm along the colonic mucosa but the introduction of current, state-of-the-art endoscopic equipment[4] and the use of mucosal dye spraying[5] may have rendered this practice redundant.

Whenever a dysplastic lesion is encountered during a surveillance colonoscopy, the management strategy for removal and follow-up is guided by several factors, including the morphology of the lesion, grade of dysplasia and presence or absence of neoplasia in the mucosa surrounding the lesion.[6] Most guidelines on IBD surveillance state that all visible lesions suspected of being neoplastic should be endoscopically removed, if possible. Additionally, biopsies should be taken from the mucosa surrounding the lesion.[7–10] These biopsies should be negative for dysplasia to refrain from performing a colectomy. It can be questioned, however, if biopsies of surrounding mucosa are still needed in this era of high definition (HD) endoscopes and chromoendoscopy.

The aim of the present study was to assess the dysplasia yield from biopsies of surrounding mucosa. Additionally, we aimed to study the clinical consequences of these biopsies in the setting of surveillance colonoscopies in IBD.

METHODS

Patient selection

Patients undergoing colonoscopic surveillance for IBD between January 2000 and January 2015 were identified from three Dutch tertiary referral centers using diagnosis treatment combinations for IBD in the respective hospital databases. The diagnosis treatment combinations are in many ways analogous to the WHO International Classification of Disease coding system. Only colonoscopies in which a random biopsy protocol or pancolonic dye-spraying was employed were considered surveillance procedures and included in this study. From 2010 onwards, chromoendoscopy has been gradually incorporated as the standard of care for IBD surveillance in the participating centers. The patients' medical records were reviewed to retrieve demographic data, IBD type, date of IBD diagnosis, maximum endoscopic disease extent, family history of CRC and a history of prior dysplasia before the studied surveillance period.

Identification of dysplastic lesions

For the observed study period all endoscopy and pathology reports were manually screened for the occurrence of colonic dysplasia. For each dysplastic lesion, location, size, morphology and grade of dysplasia were documented. Morphology of the dysplastic lesion was categorized as polypoid or non-polypoid, based on the endoscopic description. Lesions described as sessile or pedunculated polyps and Paris type 0-1 lesions were classified as polypoid. Lesions labeled as flat, non-polypoid or as mucosal irregularities were considered non-polypoid. Histopathological grade of dysplasia was categorized as low-grade dysplasia (LGD), high-grade dysplasia (HGD) or carcinoma (CRC).[11] Lesions described in the pathology report as indefinite for dysplasia (IND) were excluded. Dysplastic lesions accompanied by LGD in spatially distinct mucosa on random biopsies or LGD originating from other targeted lesions during the same colonoscopic procedure were categorized as multifocal dysplasia. The extent and grade of endoscopic inflammation was recorded and endoscopy and pathology reports were reviewed for the presence of biopsies taken in direct

proximity to the lesion. Biopsies were classified as biopsies of surrounding mucosa if both the endoscopy and pathology report provided biopsy results unequivocally traceable to a single dysplastic lesion. Biopsies described as taken directly from the outer margin of the lesions, or biopsies taken from a polypectomy scar were excluded. For each lesion, the number of individual biopsy specimens was retrieved from the pathology report. If revision of a biopsy was performed, the revised conclusion was used in the analyses.

Statistical analysis

Baseline demographic data are presented for unique patients, rather than lesions. Dichotomous outcomes are presented as the number of events with corresponding percentage, and were compared using the χ^2 method or Fisher's exact test where appropriate. Continuous data are presented as means with standard deviation (SD) or median and range and were compared by the Student *t* test or Mann-Whitney U-test according to normality. The P-value for identification of a statistically significant difference was set at 0.05. All data analyses were performed using SPSS version 21 (Armonk, NY: IBM Corp.).

RESULTS

Patients

In total, 1,065 IBD were enrolled in surveillance programs during the study period. In 196 patients (18%) a visible dysplastic lesion was found during surveillance within the studied period. For 140 dysplastic lesions (71 patients), additional biopsies of surrounding mucosa were taken. Clinical characteristics of the patients are displayed in Table 1. The majority of patients were male (63.4%). Thirty-four patients (48%) had ulcerative colitis (UC), 30 patients (42%) had Crohn's disease (CD) and 7 patients (10%) had indeterminate colitis. Mean age at the time of IBD diagnosis was 35 (±13) years and mean age at the time of dysplasia diagnosis was 56 (±12) years.

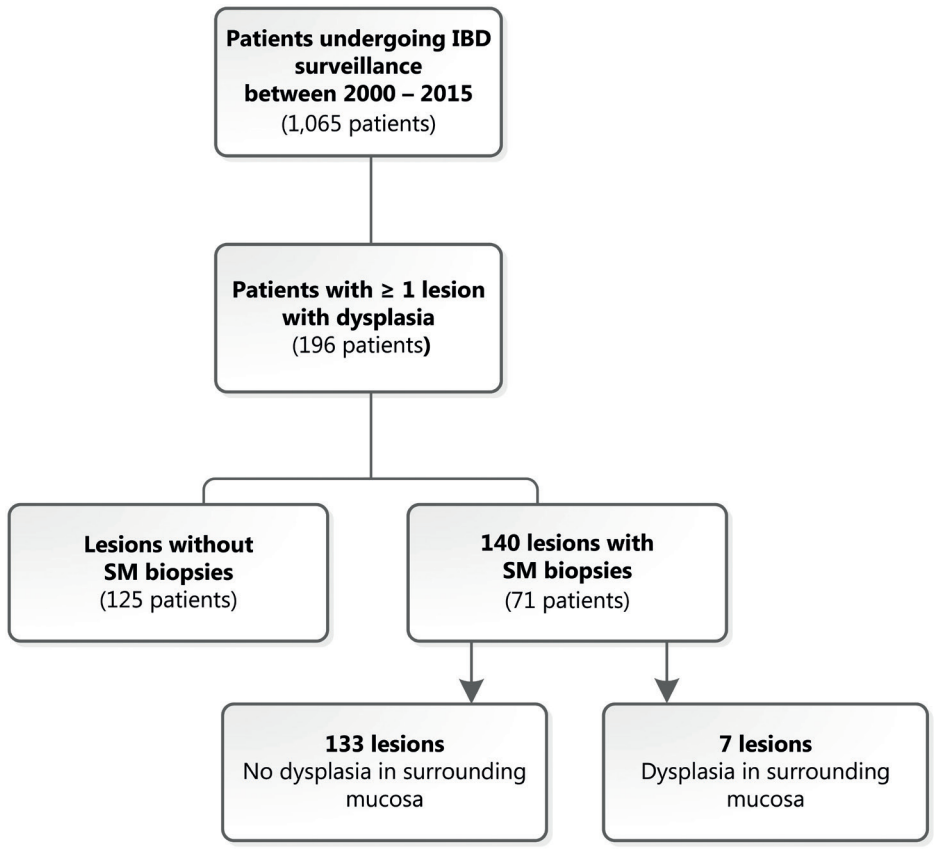


Figure 1. Flowchart for selection of cases

Table 1. Baseline characteristics for included patients

	n (%)
Number of patients	71
Male gender (%)	45 (63.4%)
Ulcerative colitis	34
- Extensive colitis	9 (26.4%)
- Left sided	24 (70.6%)
- Unknown	1 (2.9%)
Crohn's colitis	30
- Segmental colitis >50%	13 (43.3%)
- Segmental colitis <50%	15 (50.0%)
- Unknown	2 (6.7%)
Indeterminate colitis	7
- Segmental colitis >50%	1 (14.3%)
- Segmental colitis <50%	5 (71.4%)
- Unknown	1 (14.3%)
Age at first dysplasia detection, years (mean±SD)	56.0±11.9
Age at IBD diagnosis, years (mean±SD)	34.6±13.1
History of dysplasia (before surveillance)	10 (14.1%)
Postinflammatory polyps	12 (17.1%)
PSC	4 (5.7%)

Lesion with biopsies of surrounding mucosa

Table 2 displays results of the per lesion analysis for the included cases. Lesions with biopsies of surrounding mucosa had a mean size of 5.5 mm and the majority had polypoid morphology (82%). The mean number of biopsies of surrounding mucosa per lesion was 3.3 (range 1-6). In 83 cases (60%) multifocal LGD was found. Ninety-eight lesions (70%) were removed endoscopically and 12 lesions (9%) using biopsy forceps, while 30 lesions (21%) were initially only biopsied.

Table 2. Per lesion analysis, cases with surrounding mucosa biopsies

	Total	SM negative for dysplasia	SM positive for dysplasia	p-value
Number of lesions	140	133	7	-
Number of SM biopsies	3.4	3.3	3.7	0.36
Lesion size, mm (mean)	5.5	5.3	8.8	0.21
Morphology - Polypoid - Nonpolypoid	115 (82.1%) 25 (17.9%)	111 (83.5%) 22 (16.5%)	4 (57.1%) 3 (42.9%)	0.21
Histologic architecture - Adenomatous - Serrated - Other/colitis-associated	108 (77.2%) 10 (7.1%) 22 (15.7%)	104 (78.2 %) 10 (7.5%) 19 (14.3%)	4 (57.1%) - 3 (42.9%)	0.08
Location of lesion - Left colon - Transverse colon - Right colon	55 (39.3%) 32 (22.9%) 53 (37.9%)	53 (39.8%) 30 (22.6%) 50 (37.6%)	2 (28.6%) 2 (28.6%) 3 (42.9%)	0.83
Multifocal dysplasia	84 (60.0%)	81 (60.9%)	3 (42.9%)	0.49
Lesion management: **- Endoscopic removal** **- Forceps removal** **- Initially only biopsied**	98 (70%) 12 (9%) 30 (21%)	95 (71.4%) 10 (7.5%) 28 (21.1%)	3 (42.9%) 2 (28.6%) 2 (28.6%)	0.11
Endoscopic technique used: **- Chromoendoscopy** **- SD-WLE** **- HD-WLE**	53 (37.9%) 44 (31.4%) 43 (30.7%)	51 (38.3%) 42 (31.6%) 40 (30.1%)	2 (28.6%) 2 (28.6%) 3 (42.9%)	0.78

Dysplasia yield from biopsies of surrounding mucosa

Dysplasia was detected in SM biopsies in 7 out of 140 neoplastic lesions (5.0%). The rate of dysplasia per biopsy was 7 of 492 biopsies (1.4%). The 7 lesions with surrounding mucosa dysplasia were larger than the lesions without surrounding mucosa dysplasia, although this difference was not statistically significant (8.8 mm vs. 5.3 mm, p=0.21). Morphology, site, multifocality, histology and technique used were not significantly different between the groups. Characteristics of the individual cases that were positive for surrounding mucosa dysplasia are displayed in table 3.

Surrounding mucosa dysplasia rates were not found to change overtime. Surrounding mucosa dysplasia was detected in 3 out of 70 lesions in colonoscopies performed before October 2010, and in 4 out of 70 lesions detected later on (p=0.50).

Dysplasia yield from biopsies of surrounding mucosa and grade of dysplasia of colonic lesions

Four lesions with HGD were accompanied by biopsies of surrounding mucosa, 2 of which were positive for surrounding mucosa dysplasia. Both patients underwent colectomy shortly after diagnosis. Five out of 136 LGD containing lesions (3.7%) were found to have dysplasia in biopsies of surrounding mucosa. Mean time until the first follow-up endoscopy procedure was 7.8 months and the mean number of total endoscopic follow-up endoscopies was 5.5. Among patients with LGD lesions and one or more positive biopsies of surrounding mucosa, no occurrence of HGD or CRC was observed on follow-up surveillance endoscopy over a median follow-up time of 37 months (Table 3).

Table 3. Cases with surrounding mucosa biopsies containing LGD

Case	Sex, Age	Morphology	Histopathology	Within diseased colonic segment	Multifocality (spatially distinct neoplasia)	Endoscopic management	Follow-up management (follow-up duration)	Follow-up Outcome
1	F, 51	Polypoid	Tubular adenoma	Yes	Yes	Forceps removal	Surveillance (117 months)	Recurrent LGD
2	M, 51	Polypoid	LGD	Yes	Yes	Snare polypectomy	Surveillance (63 months)	No recurrence
3	M, 70	Raised (suspected DALM)	LGD	Yes	Yes	Biopsy	Lost to follow-up	-
4	F, 67	Polypoid	Tubular adenoma	No	No	Forceps removal	Surveillance (37 months)	No recurrence
5	F, 53	Irregular mucosa	Tubular adenoma	Yes	No	Biopsy	Surveillance (34 months)	No recurrence
6	M, 46	Polypoid, (suspected CRC)	HGD	Yes	No	Snare polypectomy	Colectomy; finding of residual dysplasia ('DALM') cecum.	Recurrent LGD on rectal fold
7	M, 27	Raised (suspected DALM)	Tubulovillous adenoma with HGD	Yes	Yes	Snare polypectomy	Colectomy; findings unknown	No recurrence

DISCUSSION

This is the first study evaluating the value of taking biopsies from mucosa surrounding dysplastic lesions during surveillance colonoscopies in patients with longstanding IBD in the colon. Within this retrospective cohort, 196 patients had at least 1 visible dysplastic lesions, while biopsies of surrounding mucosa were taken in only 71 patients. The dysplasia yield of these biopsies was 7 per 140 lesions (5%) and decreased to 5 per 136 lesions (3.7%) when only lesions containing LGD were analyzed. The two patients with HGD and surrounding mucosa dysplasia were treated surgically. In patients with LGD lesions and surrounding mucosa dysplasia, colectomy was not performed and a more intensive surveillance schedule was instigated. In none of these patients HGD or cancer was found during follow-up thus far.

Resection and/or intensified surveillance is considered to be the strategy of choice for dysplastic lesions in patients with IBD of the colon.[12,13] Guidelines recommend taking additional biopsies from mucosa surrounding these lesions, in order to ensure complete removal of the lesion. However, this practice is not based on solid evidence and the impact on patient outcomes has not been assessed.[3,7,8,10] Furthermore, no consensus exists on the number of biopsies and the optimal distance from the biopsy to the margin of the lesion or polypectomy site. Variations in these factors will undoubtedly impact the yield from biopsies of surrounding mucosa. In addition, these biopsies may guide clinical decision making by confirming active or previous inflammation and thereby support the diagnosis of colitis-associated dysplasia. The relevance of this point lays in the fact that colitis-associated dysplasia is thought to progress more rapidly to advanced dysplasia than conventional adenomatous lesions.[14,15] The distinction between sporadic or colitis-associated dysplasia is hampered by the absence of unequivocal histopathological and endoscopic criteria but can be aided by the confirmation of present or previous inflammation, as provided by biopsies of surrounding mucosa.[16]

Because no previous studies on the dysplasia yield from biopsies of surrounding mucosa are available, one might compare the results from our study with those from random biopsy protocol studies.[9] Van den Broek et al. performed a retrospective

analysis of 466 surveillance colonoscopies and found a neoplasia yield of 5.7% per colonoscopy for random biopsies.[17] In the per-lesion analysis, only 24 random biopsies were found to contain dysplasia (0.2%). This study supported the hypothesis of safely omitting a random biopsy protocol due to lack of clinical impact. The best way of putting our data into perspective would, however, be a comparison with the dysplasia yield of random biopsies from procedures in which concurrent visible dysplastic lesions were identified. When analyzing dysplasia yields stratified for the detection method in a per-colonoscopy analysis, the abovementioned study reported 7 colonoscopies with dysplasia in both random and targeted biopsies out of a total of 83 colonoscopies with at least one targeted dysplastic lesion (8.4%).

Due to the fact that chromoendoscopy with targeted biopsies became the preferred surveillance method during the study period, WLE (SD and HD) was gradually replaced by pancolonic dye-spraying in all participating centers. Cases of surrounding mucosa dysplasia were found in SD-WLE, HD-WLE and chromoendoscopy procedures. HD-colonoscopes, supported by the use of dye-spraying, are expected to improve the detection of dysplasia and allow for a better assessment of the lesion once detected. On the other hand, more indistinct lesions may have been missed using one of these techniques, especially if SD and not HD equipment was used, which might have led to selection bias. The small number of positive cases precludes statistically sound conclusions on this matter.

The most straightforward explanation for positive biopsies of surrounding mucosa is a limited visibility of the lesion borders (before resection) leading to residual dysplastic tissue (after resection), as displayed in Figure 2. Less distinct lesion borders may complicate both the selection of the snare size and shape, as well as placement of a snare or other devices used in endoscopic removal. Taking biopsies of surrounding mucosa will offer the endoscopist a chance of detecting residual dysplasia, which might be further concealed by hemorrhage or cauterization after resection. However, even when the margin of a lesion can be clearly distinguished and the resection is likely to be complete, histologic assessment of the surrounding mucosa is considered standard practice.

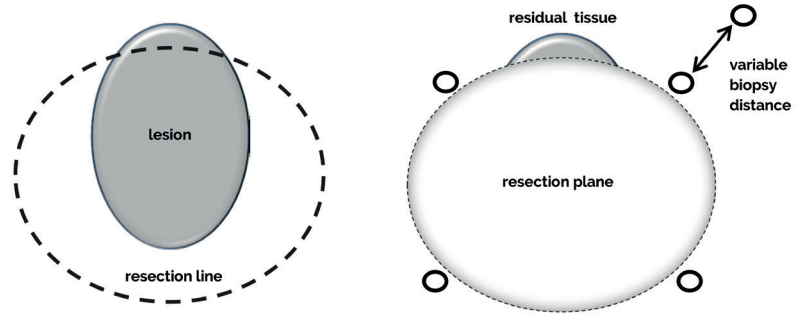


Figure 2. Schematic representation of taking biopsies of surrounding mucosa (SM) following polypectomy. Explicit recommendations on the number of biopsies, the distance of biopsies to the resection site and the timing of biopsies (before or after resection) are lacking.

There are currently no directives for the separate use of specific biopsy equipment for surrounding mucosa. It is unknown whether using the same biopsy forceps for random, semi-random and targeted biopsies in the same session may cause residual tissue of one lesion to be incorrectly allocated to another lesion.

Endoscopic removal of lesions in areas affected by colitis pose a major challenge to endoscopists and careful assessment before endoscopic resection is critical. In particular, scarring of the mucosa may lead to a piecemeal resection, which increases the risk of residual neoplastic tissue at the resection site. Whether ablative therapies, such as argon plasma coagulation or soft coagulation (using a snare tip), can safely be employed in the prevention of neoplasia recurrence in patients with IBD remains to be seen.[18]

It is important to note that our overall analysis included visible lesions containing HGD. Colectomy is considered to be the standard treatment for invisible HGD, because high rates of CRC have been reported in these patients. However, one report concluded that visible polypoid lesions containing HGD could be treated by endoscopic resection and close follow-up without surgical intervention.[19] In the present study, the majority of cases consisted of LGD lesions. Visible HGD lesions, although infrequently detected, showed surrounding mucosa dysplasia in 2 cases. This suggests a higher risk of incomplete resection for these more advanced lesions, although our study

was not designed to test this hypothesis. Currently, the optimal management of HGD in a lesion that is endoscopically visible remains ill-defined, in part because because data on the risk of cancer after endoscopic management is lacking.

Strengths of this study include the fact that a large cohort was used to identify cases. In addition, endoscopies were performed in referral centers by experienced endoscopists using state-of-the-art endoscopic equipment. Our cohort consists of patients eligible for IBD surveillance regardless of the specific IBD subtype (UC, CD or indeterminate colitis), which is in contrast to some earlier studies in which only patients with UC were included.

Our study also has some limitations. First, as this is a retrospective study, there were no standard operating procedures for sampling the surrounding mucosa. Second, biopsies of surrounding mucosa were only available for a subgroup of dysplastic lesions and the physicians' considerations to refrain from taking these additional biopsies could not be ascertained. It is possible that positive cases were missed due to misjudging of the apparent need for biopsies of surrounding mucosa, for example if the endoscopic appearance resembles that of a sporadic lesion or if the lesion is found outside of an area visibly affected by inflammation. Also, a lesion that is not well circumscribed may lower the threshold for taking additional biopsies. However, we expect that this selection bias would rather have led to an increase in the rate of dysplasia, since the endoscopist would have been more frequently inclined to take biopsies of surrounding mucosa in case of indistinct lesion borders. Nonetheless, the number of cases in which this specific guideline recommendation is adhered to is strikingly low.

We chose not to include biopsies of mucosa surrounding targeted lesions that turned out to be non-dysplastic, which would have decreased the relative number of positive biopsies in this study. Third, the retrospective nature of our study precluded systematic categorization of lesions and the exact technique of lesion removal. Moreover, not all lesions were directly removed endoscopically after taking biopsies of surrounding mucosa, which makes the interpretation of the subsequent disease course more difficult.

In summary, this study evaluated the rate of dysplasia in biopsies routinely taken from mucosa surrounding dysplastic lesions in patients undergoing IBD surveillance. The rate of surrounding mucosa dysplasia was 5.0% per lesion, which suggests that the general ability to demarcate lesion margins is good. When our results are confirmed in a larger and prospective setting, the current practice of routinely taking biopsies of surrounding mucosa may become redundant. The increasing use of HD equipment, aided by advanced imaging techniques, will likely continue to enhance visibility and reduce the need to routinely take these additional biopsies. Conversely, when relied on too heavily, negative biopsies of surrounding mucosa may lead to a false sense of security if other endoscopic features and patient characteristics are overlooked. Factors other than the presence or absence of dysplasia should be included in a consideration towards more selective use of these biopsies. Specifically, making a distinction between sporadic and colitis-associated dysplasia solely based on endoscopic features can be problematic. In any case, pre-removal assessment should include thorough cleaning of the lesion and surrounding mucosa. Additionally, targeted chromoendoscopy as well as digital image enhancing techniques can aid in the proper identification of the lesion and its borders.

The lack of clinical consequences related to taking biopsies of surrounding mucosa casts doubt on the usefulness and cost-effectiveness of this practice as a routine measure.

REFERENCES

1. Eaden, J. A., Abrams, K. R. & Mayberry, J. F. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* **48,** 526–35 (2001).
2. Bernstein, C. N., Blanchard, J. F., Kliewer, E. & Wajda, A. Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* **91,** 854–62 (2001).
3. Laine, L. *et al.* SCENIC International Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease. *Gastroenterology* **148,** 639–651.e28 (2015).
4. Subramanian, V. *et al.* Comparison of high definition with standard white light endoscopy for detection of dysplastic lesions during surveillance colonoscopy in patients with colonic inflammatory bowel disease. *Inflamm. Bowel Dis.* **19,** 350–5 (2013).
5. Subramanian, V., Mannath, J., Ragunath, K. & Hawkey, C. J. Meta-analysis: the diagnostic yield of chromoendoscopy for detecting dysplasia in patients with colonic inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **33,** 304–12 (2011).
6. Rutter, M. D. & Riddell, R. H. Colorectal dysplasia in inflammatory bowel disease: a clinicopathologic perspective. *Clin. Gastroenterol. Hepatol.* **12,** 359–67 (2014).
7. Cairns, S. R. *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* **59,** 666–689 (2010).
8. Kornbluth, A. & Sachar, D. B. Ulcerative Colitis Practice Guidelines in Adults: American College of Gastroenterology, Practice Parameters Committee. *Am. J. Gastroenterol.* **105,** 501–523 (2010).
9. Marion, J. F. & Sands, B. E. The SCENIC Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease: Praise and Words of Caution. *Gastroenterology* **148,** 462–467 (2015).
10. Annese, V. *et al.* European evidence based consensus for endoscopy in inflammatory bowel disease. *J. Crohns. Colitis* **7,** 982–1018 (2013).
11. Riddell, R. H. *et al.* Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. *Hum. Pathol.* **14,** 931–68 (1983).
12. Wanders, L. K. *et al.* Cancer risk after resection of polypoid dysplasia in patients with longstanding ulcerative colitis: a meta-analysis. *Clin. Gastroenterol. Hepatol.* **12,** 756–64 (2014).
13. Rubin, P. H. *et al.* Colonoscopic polypectomy in chronic colitis: conservative management after endoscopic resection of dysplastic polyps. *Gastroenterology* **117,** 1295–300 (1999).
14. Itzkowitz, S. Colon carcinogenesis in inflammatory bowel disease: applying molecular genetics to clinical practice. *J. Clin. Gastroenterol.* **36,** S70-4-6
15. Choi, C.-H. R. *et al.* Low-Grade Dysplasia in Ulcerative Colitis: Risk Factors for Developing High-Grade Dysplasia or Colorectal Cancer. *Am. J. Gastroenterol.* **110,** 1461–71 (2015).
16. Bernstein, C. N. ALMs versus DALMs in ulcerative colitis: polypectomy or colectomy? *Gastroenterology* **117,** 1488–92 (1999).
17. van den Broek, F. J. C. *et al.* Random biopsies taken during colonoscopic surveillance of patients with longstanding ulcerative colitis: low yield and absence of clinical consequences. *Am. J. Gastroenterol.* **109,** 715–22 (2014).
18. East, J. E., Toyonaga, T. & Suzuki, N. Endoscopic management of nonpolypoid colorectal lesions in colonic IBD. *Gastrointest. Endosc. Clin. N. Am.* **24,** 435–45 (2014).
19. Blonski, W. *et al.* High-grade dysplastic adenoma-like mass lesions are not an indication for colectomy in patients with ulcerative colitis. *Scand. J. Gastroenterol.* **43,** 817–20 (2008).

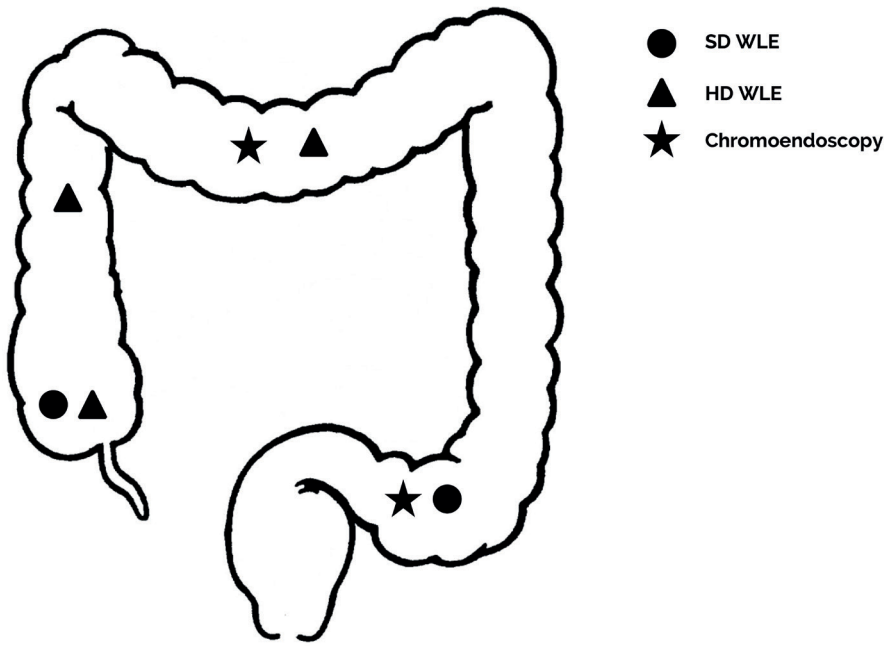
SUPPLEMENTARY MATERIALS

Supplementary table 1. Excerpts of guidelines regarding sampling of surrounding mucosa

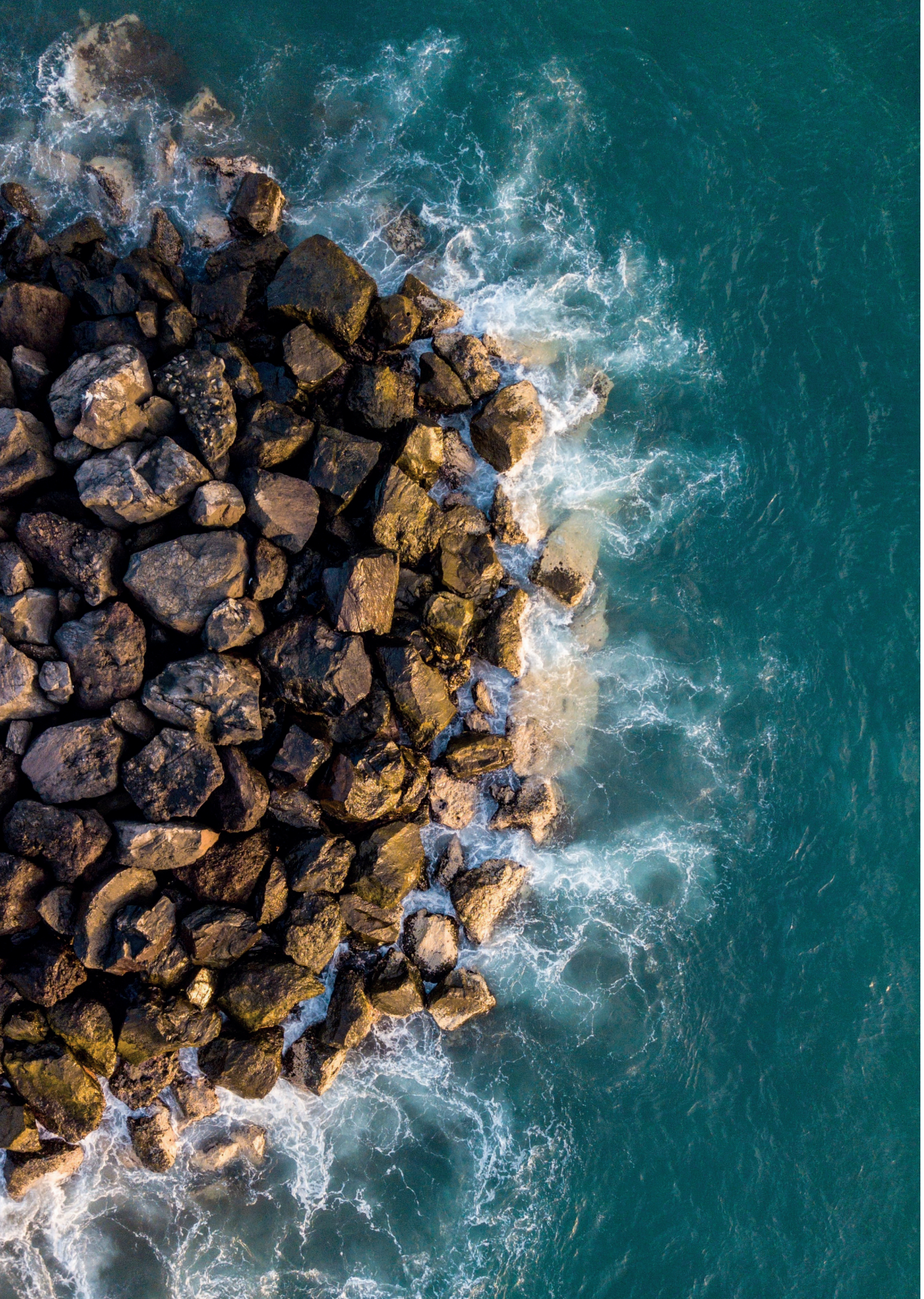
BSG guideline (update 2010) [7]	"It is essential to biopsy the flat mucosa surrounding any dysplastic polyp to assess the extent of disease (as it may not be apparent macroscopically) and also to assess whether there is any dysplasia in the surrounding flat mucosa. If a dysplastic polyp occurs in an area proximal to the microscopic level of inflammation, with no dysplasia in flat mucosa, it can be regarded as a sporadic adenoma and treated accordingly."
SCENIC statement; joint AGA & ASGE consensus (2015) [3]	"The term endoscopically resectable indicates that distinct margins of the lesion could be identified, the lesion appears to be completely removed on visual inspection after endoscopic resection, histologic examination of the resected specimen is consistent with complete removal, and biopsy specimens taken from mucosa immediately adjacent to the resection site are free of dysplasia on histologic examination."
ECCO statement (2013) [10]	"If the polypectomy is confirmed complete by histology, biopsies obtained from the flat mucosa immediately adjacent to the polypectomy site show no dysplasia and no dysplasia is found elsewhere in the colon, a careful colonoscopic follow-up preferably with chromoendoscopy at 3 months before reverting to annual surveillance is recommended, because at least half of such patients may developed further lesions. If the lesion is not resectable, or is associated with dysplasia in the adjacent mucosa, then colectomy is indicated due to the high risk of concomitant CRC."
ACG (2010) [8]	"If the lesion is resected in its entirety by colonoscopic polypectomy and if no dysplasia is found in the adjacent flat mucosa or anywhere else in the colon, long-term follow-up has not found an increased risk of cancer in these cases, suggesting that vigilant follow-up surveillance colonoscopy may suffice"

Supplementary table 2 – Baseline characteristics for all patients with ≥1 lesion containing dysplasia

	Total	SM biopsies	No SM biopsies	p-value
Number of patients	196	71	125	-
Male gender (%)	119 (60.7%)	45 (63.4%)	74 (59.2%)	0.39
Ulcerative colitis	116	34	82	**0.03**
- Extensive colitis	50 (43.1%)	9 (26.4%)	41 (50.0%)	
- Left sided	57 (49.1%)	24 (70.6%)	33 (40.2%)	
- Unknown	9 (7.8%)	1 (2.9%)	8 (9.8%)	
Crohn's colitis	69	30	39	0.16
- Segmental colitis >50%	40 (58.0%)	13 (43.3%)	27 (69.2%)	
- Segmental colitis <50%	24 (34.8%)	15 (50.0%)	9 (23.1%)	
- Unknown	5 (7.2%)	2 (6.7%)	3 (7.7%)	
Indeterminate colitis	11	7	4	0.21
- Segmental colitis >50%	4 (36.4%)	1 (14.3%)	3 (75.0%)	
- Segmental colitis <50%	6 (54.5%)	5 (71.4%)	1 (25.0%)	
- Unknown	1 (9.1%)	1 (14.3%)	-	
Age at first dysplasia detection, years (mean±SD)	55.4±11.7	56.1±11.9	55.0±11.5	0.53
Age at IBD diagnosis, years (mean±SD)	32.9±14.1	34.6±13.1	31.9±14.6	0.20
History of dysplasia (before surveillance)	22 (12.0%)	10 (14.1%)	12 (10.5%)	0.31
Postinflammatory polyps	53 (28.8%)	12 (17.1%)	41 (35.3%)	**0.01**
PSC	16 (8.4%)	4 (5.7%)	14 (11.5%)	0.14



Supplementary figure 1 – Location of lesions with positive SM biopsies, according to endoscopic technique used to detect lesion



CHAPTER 8

Endoscopic resection of large dysplastic colorectal lesions in patients with Inflammatory Bowel Disease

Submitted

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ABSTRACT

Background: Patients with longstanding colitis have an increased risk of developing colorectal cancer (CRC). Endoscopic resection of small dysplastic colorectal lesions is currently accepted in IBD patients but data on the safety, feasibility and follow-up of endoscopic resection of large (≥20mm) neoplastic colorectal lesions in patients with IBD are virtually non-existent.

Methods: We performed a retrospective analysis of endoscopic procedures performed between August 2012 and March 2017 in two tertiary referral centers in the Netherlands. Inclusion criteria were: age above 18 years, histopathologically confirmed IBD, lesion size ≥20mm, non-pedunculated morphology, lesion resected by endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD).

Results: Nineteen patients were included (58% male; median age 60 years). Eleven patients had ulcerative colitis, 6 patients had Crohn's disease and 2 patients had indeterminate colitis. Three patients (16%) had evidence of mild active disease during the procedure.

All neoplastic lesions were located in an area previously affected by colitis. The median diameter of the lesions was 30mm (range 20-100mm). Resection technique included EMR in 12 cases (11 piecemeal EMR; 1 en-bloc EMR), ESD in 6 cases (6 en-bloc resections) and 1 piecemeal hybrid ESD. All resections were macroscopically complete (i.e. no visible remnant tissue). In 5 out of 6 ESDs, submucosal fibrosis was reported as a factor complicating the procedure. In 9 out of 13 EMRs, suboptimal lifting was reported.

Follow-up data were available in 18 patients (median 18 months). In 1 case, recurrent LGD at the scar was observed. Two patients were found to have synchronous lesions (1 CRC, 1 SSA). One patient developed a metachronous lesion (>1y) containing HGD and in three patients sporadic adenomas (LGD) were removed on follow-up.

Conclusion: Endoscopic resection of large colorectal lesions in patients with IBD is feasible and safe, although it can be technically demanding due to submucosal fibrosis. A detailed baseline colonoscopy should rule out synchronous lesions, whereas close follow-up is warranted to assess for metachronous lesions.

INTRODUCTION

Patients with longstanding Inflammatory Bowel Disease (IBD) involving the colon are at increased risk of developing colorectal cancer (CRC).[1,2] Regular colonoscopic surveillance is performed as a means to prevent CRC-related mortality. With improved endoscopic imaging and resection techniques over the years, the management of dysplasia in IBD patients has changed significantly. It is now acknowledged that most visible lesions encountered in IBD patients are well delineated and can be safely resected endoscopically, as long as the resection is complete.[3–5]

The current body of evidence on endoscopic resection of dysplasia in IBD patients mainly encompasses follow-up data after polypectomy of small lesions.[6,7] To date, very little data are available on endoscopic resection of large (≥ 20 mm) lesions, since these patients are often referred for surgery.[8,9]

Endoscopic mucosal resection (EMR) is a straightforward technique, yet carries a risk of incomplete resection if en-bloc resection fails. This is particularly true in patients with IBD, due to submucosal fibrosis and concurrent inflammation. In this setting, endoscopic submucosal dissection (ESD) may ensure complete removal and adequate histopathological assessment.

The aim of this study was to investigate the feasibility and safety of endoscopic resection of large (≥20 mm) non-pedunculated colorectal lesions in patients with IBD.

MATERIALS AND METHODS

Patient selection

We performed a retrospective analysis of endoscopic procedures performed between August 2012 and March 2017 in two tertiary referral centers in the Netherlands.

Inclusion criteria were: 1) Established IBD (ulcerative colitis, Crohn's colitis or IBD-Unclassified), confirmed by histopathology; 2) Non-pedunculated colorectal neoplastic lesion ≥ 20 mm identified during screening, surveillance or diagnostic colonoscopy in an area affected by colitis; 3) ≥18 years of age.

Patients were excluded if one of the following criteria was met: 1) Active IBD, as defined by a total Mayo endoscopy subscore ≥ 2 points (moderately-to-severely active disease on colonoscopy); 2) Optical diagnosis of ≥ T1 CRC-sm1 polyp, as determined by endoscopic findings (Hiroshima type C3 or non-lifting sign Kato IV); 3) Synchronous lesions demanding surgical resection (synchronous dysplasia/CRC, non-circumscribed lesion).

Endoscopic procedure

Patients were treated with endoscopic resection according to the current standards of care. Procedures were performed under conscious sedation with intravenous administration of midazolam and fentanyl or with intravenous administration of propofol and alfentanyl. All colonoscopies were performed with a high-resolution videoendoscope. Follow-up colonoscopies were planned after 3-6 months to assess for residual disease at the scar according to the surveillance guidelines of the participating centers.

EMR

A 0.9% saline solution or succinylated gelatine together with dye was used as injection fluid (mixture with 1:100.000 adrenaline was optional). Marking of the periphery of the lesion with coagulation was allowed to optimize the attempt of an en-bloc or R0-resection. After passing the snare through the channel and opening it around the

lesion, the snare was snugged around the lesion and pulled. Cautery was then applied to resect the lesion. Only when en-bloc resection was not feasible, the endoscopist aimed to perform the resection in a piecemeal fashion (pEMR) in as few pieces as possible. Adjunct therapy with either tipping with the snare using forced coagulation or treatment with argon plasma coagulation was performed when remnant tissue was suspected.

ESD / hESD

Three endoscopists (LM, PD, AK) with extensive experience (>50 ESD procedures) performed the ESDs. A 0.9% saline solution or succinylated gelatine together with dye was used as the lifting fluid. First, a circumferential incision was made using an ESD knife on a distance of 2-5 mm from the margin of the lesion. The endoscopist could optionally perform the resection using the hybrid ESD (hESD) technique. This hESD technique consists of a circular incision around the lesion, with partial dissection in the submucosal layer that is sufficient to capture it with a snare in a single piece. Adjunct therapy with either tipping with the snare using forced coagulation or treatment with argon plasma coagulation was only performed when remnant tissue was suspected.

Histopathology

In case of ESD, the resected specimen was pinned on a paraffin, rubber or cork sheet so that the mucous membrane surrounding the lesion was evenly flattened and the mucous membrane surface could be observed.

Histological diagnosis was carried out in accordance with the Vienna classification by the pathologist of the center in which the resection is performed.[10] Incomplete (R1) resection was defined as tumor infiltration of the margins and/or if infiltration could not be determined because of coagulation artefacts, as in piecemeal resection.

Ethical approval

This study was conducted in accordance with the declaration of Helsinki and in accordance to the Dutch Medical Research Involving Human Subjects Act (WMO).

Table 1. Patient demographics

	n=19 (%)
Male	11 (58%)
Age, median, years	60 (49-84)
IBD subtype	
Ulcerative colitis	11 (58%)
Crohn's disease	6 (32%)
IBD-U	2 (10%)
IBD duration, median, years	24 (1-53)
Mild active inflammation during endoscopic resection	3 (16%)
Referral	5 (26%)
Previous colonoscopic surveillance	17 (89%)
PSC	2 (11%)

RESULTS

In total, 19 IBD patients were identified in whom an endoscopic resection of a neoplastic lesion >20mm was performed. Patient demographics are displayed in Table 1. A majority of patients was male (58%), the median age was 60 years. Eleven patients had ulcerative colitis, 6 patients had Crohn's disease and 2 patients had indeterminate colitis. Three patients had evidence of mild active disease during the procedure. A majority of patients had undergone endoscopic surveillance beforehand, leading to the detection of the lesion.

Lesion characteristics are shown in Table 2. All neoplastic lesions were located in an area previously affected by colitis. The median diameter of the lesions was 30mm (range 20-100mm). Resection technique included EMR in 12 cases (11 piecemeal EMRs; 1 en-bloc EMR), ESD in 6 cases (6 en-bloc resection) and 1 hybrid ESD (en-bloc resection). In all procedures, the resection was considered macroscopic complete (i.e. no visible remnant tissue).

In 5 out of 6 ESDs, submucosal fibrosis was reported as a factor complicating the procedure. In 9 out of 13 EMRs, suboptimal lifting was reported. One perforation occurred (5%) after EMR, which required a second endoscopic procedure with

placement of an over-the-scope clip. This patient recovered clinically following the procedure. One case of stenosis occurred 3 months after ESD, which was treated using balloon dilatation. Intra-procedural bleeding was treated endoscopically using coagulation or endoscopic clipping where necessary. No post-procedural bleeding was reported.

Histopathology revealed 6 cases of high-grade dysplasia, 12 cases of low-grade dysplasia and 2 sessile serrated lesions.

Table 2. Lesion characteristics

	n=19 (%)
Lesion diameter, median, mm (range)	30 (20-100)
Morphology	
0-Is	3 (16%)
0-IIa	10 (53%)
0-IIb	2 (10%)
0-IIa + 0IIb	3 (16%)
0-IIa + 0IIc	1 (5%)
Lesion location	
Ascending/transverse colon	8 (42%)
Descending colon	6 (32%)
Rectum	5 (26%)
Lesion within area affected by colitis	19 (100%)
Endoscopic technique	
EMR / en-bloc	12 / 1
ESD / en-bloc	6 / 6
hESD / en-bloc	1 / 1
Histology	
LGD	11 (58%)
HGD	6 (32%)
Serrated lesion	2 (10%)
Submucosal fibrosis or bad lifting reported in complicating procedure	13 (68%)

Table 2. Continued

	n=19 (%)
Follow-up	
No dysplasia	11 (58%)
Metachronous dysplasia	4 (32%)
Synchronous (<12mo) dysplasia	2 (10%)
Recurrence (at scar)	1 (10%)
No follow-up	1 (5%)

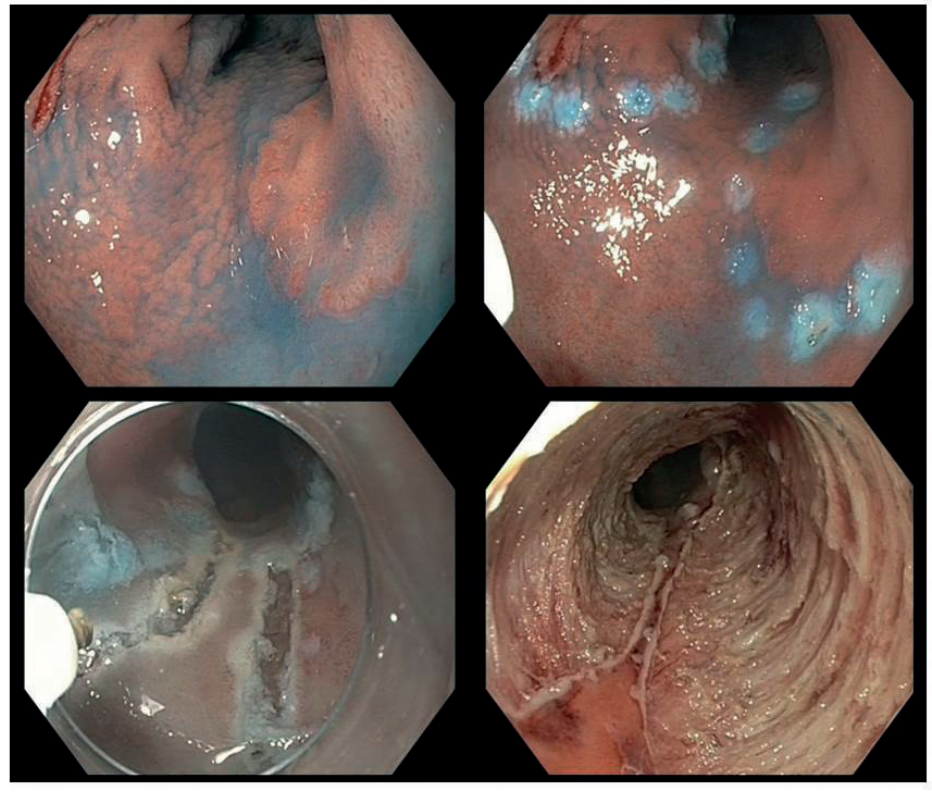

Figure 1. Endoscopic images of a large neoplastic lesion (involving 2/3 of colonic circumference) removed with ESD.

Follow-up

Endoscopic follow-up data were available for 18 patients (median follow-up 9 months). In 1 case, recurrent LGD at the scar was observed. Two patients were found to have synchronous lesions: in 1 patient, CRC associated with a stricture

was detected and this patient subsequently underwent colectomy. In the second patient with a synchronous lesion, a serrated lesion was detected and removed endoscopically after 6 months. One patient developed metachronous HGD (>1y) and underwent subsequent colectomy. Three patients were found to have metachronous LGD on follow-up (all adenomatous lesions), which was both managed and followed endoscopically.

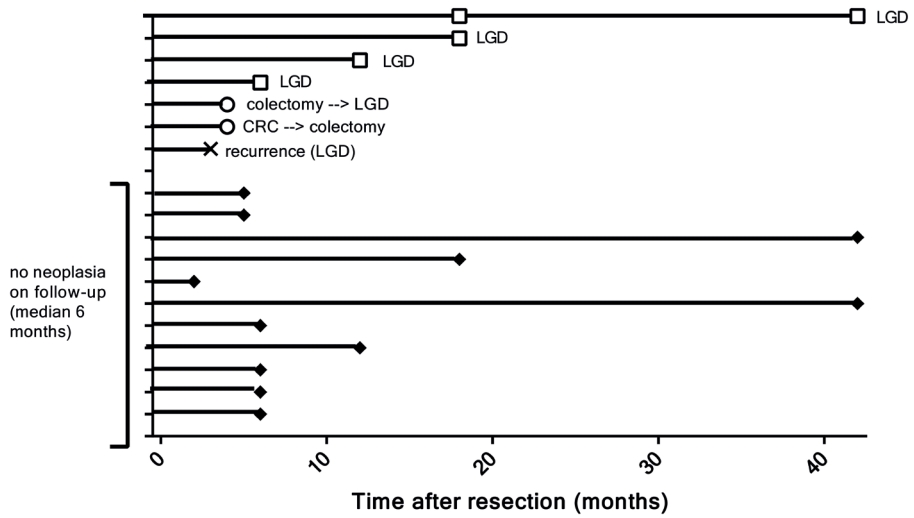

Figure 2. timeline of follow-up

DISCUSSION

In this case-series, we report our experiences with the endoscopic resection of large (≥2 cm) neoplastic lesions arising in areas affected by colitis in patients with IBD. Over a period of nearly 5 years, we identified 19 patients in whom this procedure had been performed and in these patients this proved to be technically feasible and safe. Endoscopic follow-up was available 18/19 patients. In one patient recurrence (LGD) was observed. In two patients an advanced neoplastic lesion (one case of CRC and one case of HGD) resulted in colectomy.

The detection and management of colonic neoplasia in patients with IBD is known to be challenging. The recent introduction of advanced endoscopic techniques

broadens the possibilities for IBD patients with precancerous lesions and may offer an alternative to colectomy. Currently, any neoplastic lesion detected in an area affected by IBD should be assessed for endoscopic removal.[3] Irrespective of size or morphologic features, en-bloc resection should be the intent. Neoplastic lesions will often be detected during a surveillance procedure and endoscopists might be inclined to remove this lesion directly via EMR. Unfortunately, in case of a large or non-pedunculated lesion, EMR might result in piecemeal resection, increasing the risk of residual tissue (recurrence) and hampering histopathological assessment. For achieving en-bloc resection, especially in the setting of IBD, ESD may be more appropriate.

The effects of persistent inflammatory activity on the (sub-)mucosa may hamper the ease and effectiveness of endoscopic resection in several ways. An obvious example is submucosal fibrosis, as a result of previous inflammation or a previous attempt at endoscopic removal. The effect of fibrosis –for example, an inability to adequately lift the lesion- can be observed in both EMR and ESD. In case of the latter, this may lead to slower speed of resection or the need to convert to EMR. Active inflammation during the procedure itself may also impede endoscopic resection, e.g. by blurring lesion demarcations. Notably, the morphology of dysplastic lesions is more often flat in an area affected by colitis, further complicating the resection. All operators had wide experience with endoscopic resections, but still encountered marked technical challenges in the procedures included in this study.

Additionally, demarcation of lesion borders is often more challenging in IBD colitis, especially in case of active inflammation or severe scarring of the mucosa .

Although a considerable amount of studies has been published on the risk of neoplasia following endoscopic resection of small lesions in IBD, the number of studies addressing the endoscopic approach of large lesions is limited.

In the first case-series evaluating lesions of this size-category, Iacopini et al. described successful endoscopic resection in 10 patients with lesions ranging from 20-50mm (8 en-bloc ESDs and 2 pEMRs).[8] On follow-up, 2 patients (20%) had residual LGD at the

resection site, and 3 patients (30%) were found to have metachronous LGD. Another case-series, published in 2017, described ESD in 32 UC patients, 19 of whom had lesions ≥20 mm in size.[9] At follow-up, 1 patient was found to have recurrence, whereas 3 patients were found to have metachronous lesions. Interestingly, in this Japanese-British series, 4 cases of T1 CRC were detected.

A larger lesion size not only increases the risk of complications and recurrence of neoplasia, but also increases the risk of the lesion containing malignancy. In our study, none of the lesions included in this study was found to be invasive (T1) after resection. Currently, in patients with colitis-associated cancer, even if invasion is limited to the submucosa, proctocolectomy is recommended. Another reason to consider colectomy, rather than endoscopic resection, is a finding of synchronous or metachronous neoplasia. Whether endoscopic resection is a durable strategy in case of repeated findings of neoplasia is less clearly defined. If endoscopic resection is the new paradigm, the number of times a patient with repeated findings of LGD can be safely endoscopically managed should be studied in more detail.

We acknowledge that lesions presented in this report have specifically been considered amenable to endoscopic resection. Therefore, our results may not be generalizable to all large colitis-associated dysplastic lesions.

In summary, endoscopic removal of large dysplastic lesions in patients with colonic IBD is feasible and safe in selected cases, despite the presence of submucosal fibrosis in a considerable number of patients. The frequent occurrence of neoplastic lesions after complete resection warrants a course of close endoscopic surveillance, at least in the first 1-2 years. The need for pre-resection endoscopy (chromoendoscopy) should also be emphasized, as this group of patients is at risk of having synchronous lesions elsewhere in the colon.

REFERENCES

1. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
2. Choi, C.-H. R. *et al.* Forty-Year Analysis of Colonoscopic Surveillance Program for Neoplasia in Ulcerative Colitis: An Updated Overview. *Am. J. Gastroenterol.* **110,** 1022–34 (2015).
3. Laine, L. *et al.* SCENIC International Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease. *Gastroenterology* **148,** 639–651.e28 (2015).
4. Magro, F. *et al.* Third European Evidence-Based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J. Crohn's Colitis* (2017). doi:10.1093/ecco-jcc/jjx008
5. Rutter, M. D. & Riddell, R. H. Colorectal dysplasia in inflammatory bowel disease: a clinicopathologic perspective. *Clin. Gastroenterol. Hepatol.* **12,** 359–67 (2014).
6. Wanders, L. K. *et al.* Cancer Risk After Resection of Polypoid Dysplasia in Patients With Longstanding Ulcerative Colitis: A Meta-analysis. *Clin. Gastroenterol. Hepatol.* **12,** 756–764 (2014).
7. Odze, R. D., Farraye, F. A., Hecht, J. L. & Hornick, J. L. Long-term follow-up after polypectomy treatment for adenoma-like dysplastic lesions in ulcerative colitis. *Clin. Gastroenterol. Hepatol.* **2,** 534–41 (2004).
8. Iacopini, F. *et al.* Curative endoscopic submucosal dissection of large nonpolypoid superficial neoplasms in ulcerative colitis (with videos). *Gastrointest. Endosc.* **82,** 734–8 (2015).
9. Suzuki, N., Toyonaga, T. & East, J. E. Endoscopic submucosal dissection of colitis-related dysplasia. *Endoscopy* (2017). doi:10.1055/s-0043-114410
10. Schlemper, R. J. *et al.* The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* **47,** 251–5 (2000).

CHAPTER 9

Putting evidence into practice: IBD surveillance, chromoendoscopy and future directions

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INTRODUCTION

The cumulative risk of colorectal cancer (CRC) in patients with ulcerative colitis or Crohn's colitis is estimated to be 5% after a disease duration of ≥20 years.[1] This risk is modulated by several clinical and endoscopic risk factors. (Table 1) In order to detect and treat neoplastic lesions in these patients, guidelines advocate endoscopic surveillance[2–5], although the level of evidence for its effectiveness is low.[6] Chromoendoscopy (CE) has been endorsed by clinical society guidelines as the preferred technique, based on recent evidence.[7] Although the logistics of CE are relatively straightforward and there is no steep learning curve for the technique[8], the uptake of CE in daily practice has not been uniform. Whether this can be attributed to time constraints, lack of training, lack of interest or awareness, or lack of reimbursement is unclear.

Herein, we provide practical advice on performing IBD surveillance. We touch upon common difficulties and outline unresolved questions on the matter.

Selection of patients for surveillance

The only objective measures suggested for selecting patients for surveillance strategies are disease extent, disease duration and the presence of PSC. Inflammation is known to correlate with the risk of dysplasia, yet there is no scoring system for the amount of cumulative inflammation in the colon, which is considered to be the main driver for the increased cancer risk.

In UC, at least left sided colitis warrants surveillance; In CD, it is more challenging to ascertain the exact extent of (previously) inflamed mucosa, due to an often discontinuous distribution of inflammation. In this case, involvement of at least one third of the colon is mostly considered an indication for surveillance.[2]

While surveillance is recommended to begin at 8 to 10 years of disease duration, it is uncertain whether disease onset should be clocked from the time of first diagnosis or time of first symptoms. Since a considerable number of patients develop CRC within 8 years after the onset of IBD[9], we use the latter to calculate this time interval.

We base surveillance intervals on the 3-tier system reported in European guidelines[4,5], with a high-risk (1-year interval), intermediate risk (3-year interval) and low-risk (5-year interval) group. This interval range is narrower (1-3 years) in the North-American guidelines.[2] The evidence for these stratifications is limited, however.

Table 1. Risk factors for dysplasia in chronic colitis

Risk factors
- Disease duration
- Disease extent
- Disease severity
- Past dysplasia or indefinite for dysplasia
- PSC
- Family history of CRC
Endoscopic factors associated with risk
- Active disease
- Stricture (UC)
- Post-inflammatory polyps (pseudopolyps)
- Tubular appearance of colon (loss of colonic haustration)

What is the evidence for chromoendoscopy

CE enhances the surface and crypt pattern of the mucosa, as well as lesion borders, and identifies more patients with dysplasia compared to standard-definition white light endoscopy (SDWLE). [7] The superiority of CE has not been uncontested. A recent randomized controlled trial showed similar dysplasia detection rates for NBI and CE, whilst CE required a longer withdrawal time.[10] A retrospective study examining the implementation of CE in a real life setting also found no significantly increased neoplasia detection using CE compared to white-light endoscopy.[11] Of note, additional benefit of CE over high definition white light endoscopy (HDWLE) is currently under investigation. For patients with chronic colitis at average risk, surveillance with HDWLE may be all that is necessary. More important than CE over HDLWE is a very careful endoscopic surveillance, regardless of what technique is used.

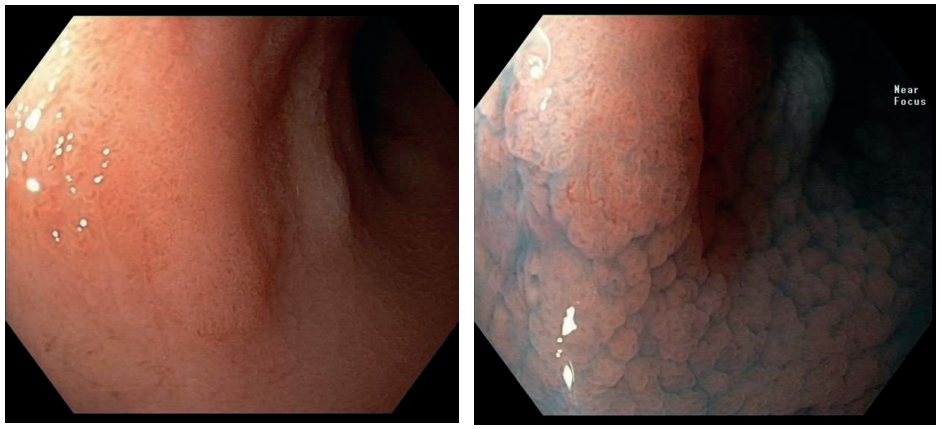


Figure 1. Endoscopic image of a large nonpolypoid lesion located in the rectum before (a) and after (b) application of dye. This lesion was later removed by endoscopic submucosal dissection (ESD).

How to perform chromoendoscopy

The SCENIC statement provides an excellent description of the practical aspects of the technique.[3] Using either the water jet channel or a spray catheter, circumferential and preferably anti-gravity (opposite to the fluid collections) application of the dye is followed by reinsertion, suction of surplus fluid and careful inspection of the segment.

Superiority of one dye over another (methylene blue or indigo carmine), or of the mode of administration, has not been explored. Indirect methods of dye administration (modified release tablets or dye mixed with polyethylene glycol) are under investigation, but currently not advised.

Biopsies

Due to a negligible diagnostic yield, now substantiated in prospective studies[12,13], we no longer routinely take random biopsies for the detection of dysplasia. We do however retain a random biopsy protocol in patients with PSC or previous neoplasia. In addition, we take 2 non-targeted biopsies per segment in order to establish histologic disease activity and extent.

Optimizing the surveillance colonoscopy

Optimal bowel preparation is an absolute prerequisite. When ordering the colonoscopy, it may be of value to check if patients have had inadequate preparation before, and adjust the type or amount of prep fluid accordingly. It is unknown how poor the dysplasia detection rate is, if there is a suboptimal preparation. Although the risk of developing interval CRC in IBD is considered limited, some cases can likely be attributed to inadequate bowel preparation.[14] We usually schedule an early repeat colonoscopy in patients with a Boston Bowel Preparation Scale of 0 or 1 in any colon segment.

If a patient's disease is not in remission, this should be resolved by adjustment of medical therapy before the surveillance takes place. Active inflammation limits dysplasia detection rate (to what extent is not known) and disrupts dye application and absorption. When characteristics of inflammation suggest a MAYO endoscopic score greater than 1, dye application may be futile and an early repeat endoscopy after remission induction would be a better strategy. Fecal calprotectin can be a helpful biomarker in verifying remission.[15]

Notably, certain high-risk patients require extra vigilance: patients with previous dysplasia, concurrent PSC, ongoing inflammation, a family history of CRC or concurrent genetic susceptibility. A referral to an endoscopist experienced in CE may be warranted in these high-risk patients.

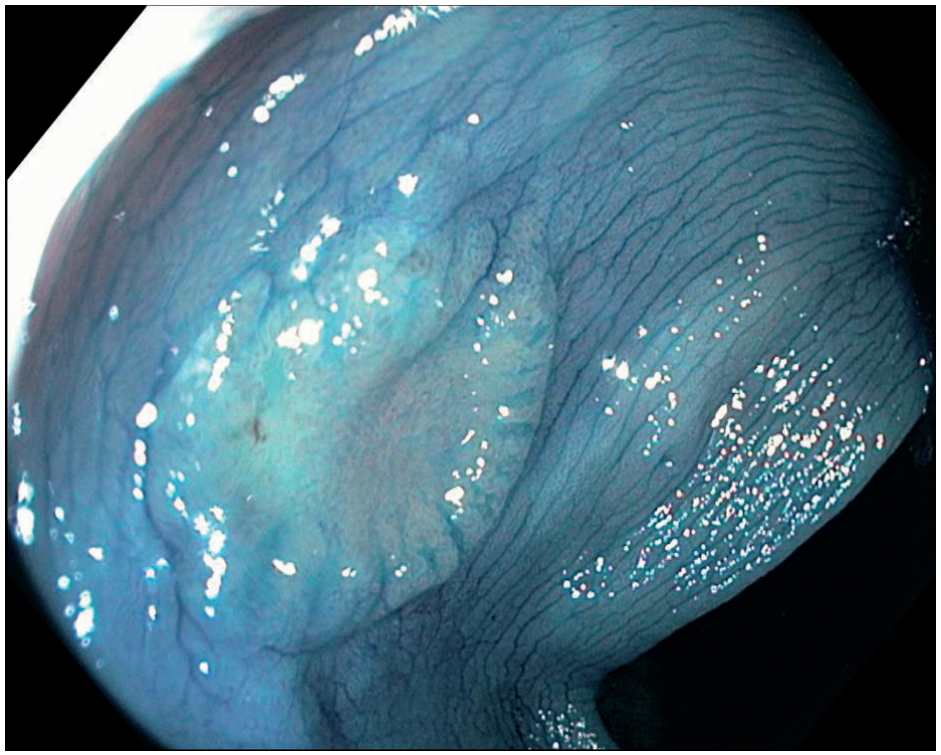


Figure 2. Endoscopic image of a nonpolypoid lesion located in the transverse colon. This lesion was removed by endoscopic mucosal resection (EMR)

When assessing a suspected lesion

Systems for optical diagnosis have not been designed for dysplasia in the setting of IBD. For the endoscopist, differentiating post-inflammatory polyps or areas of patchy inflammation from dysplasia is often challenging. If, after careful inspection, a suspicion of neoplasia remains, endoscopic removal or biopsy (+tattoo) is warranted.

Planning further follow-up if dysplasia is found

In IBD surveillance, clinicians have had a tendency to categorize dysplasia into a high-risk (colitis-associated) and a low-risk pathway. Although a range of features may suggest such a distinction (High-risk: nonpolypoid morphology, within area of inflammation, p53+. Low risk: polypoid morphology, adenoma-like, p53-), there are no

reliable criteria to stratify lesions based on endoscopic or histological characteristics. Therefore, we discuss dysplasia management in a multidisciplinary team consisting of an expert gastroenterologist, an expert GI-pathologist and GI-surgeon. Moreover, we highly value the involvement of the patient in the decision-making process.

Table 2. Tips/tricks for chromoendoscopy

Tips / tricks for chromoendoscopy
- Schedule additional time (about 10 minutes per procedure), for chromoendoscopy. - Remove all prep fluid and mucus during introduction, before dye spraying - Reinsertions after dye application: up to previous landmarks or point of previous biopsy. We routinely take 2 non-targeted biopsies per segment to assess disease activity and extent, which may also serve as landmarks. - Spray dye contra-gravity (opposite pools of excess fluid) to facilitate complete application - Spasmolytic use may be used if peristalsis hampers visualization of the mucosa (i.v. buscopan 20mg)

For small polypoid lesions, completely removed by en-bloc resection, we schedule a follow-up surveillance colonoscopy after 1 year.[3] In complex clinical scenarios (multifocality, incomplete resection, recurrent dysplasia, invisible dysplasia) the risk of continued surveillance should be weighed against the risk of colectomy, which can pose a dilemma for both the physician and patient. Invisible dysplasia, though rare, should be confirmed by a GI pathologist and the patient referred for chromoendoscopy, but surgery should be highly considered.

Large nonpolypoid lesions can pose a particular problem and have long been considered a clear indication for surgery. Experience in endoscopic resection of these lesions is limited but growing. In our view, these procedures should only be performed by expert endoscopists (proficient in ESD, EMR and full thickness resection). A damaged colon often harbors significant submucosal fibrosis, which makes mucosal resection all the more demanding. In the first place, we counsel patients extensively on the clinical scenario and risk of future neoplasia when delaying surgery. Additionally, we only proceed with endoscopic removal of difficult lesions if we have achieved good visualization of the whole colon to exclude multifocality. After complete endoscopic removal, we schedule follow-up colonoscopies at 3-6

months. Once dysplasia has been identified (unless clearly a sporadic adenoma), the next surveillance colonoscopy should be performed with CE.

Continuing the surveillance track

Follow-up should be personalized based on the risk stratification outlined in the available regional guidelines. Reasons for discontinuing surveillance are not explicitly stated, but this subject may come up in treating, for example, old patients who have repeatedly been free from dysplasia. If, based on the patient's age or comorbidity, the life expectancy is estimated to be limited, we usually allow for discontinuation of surveillance.

Table 3. Research agenda for dysplasia surveillance in chronic colitis

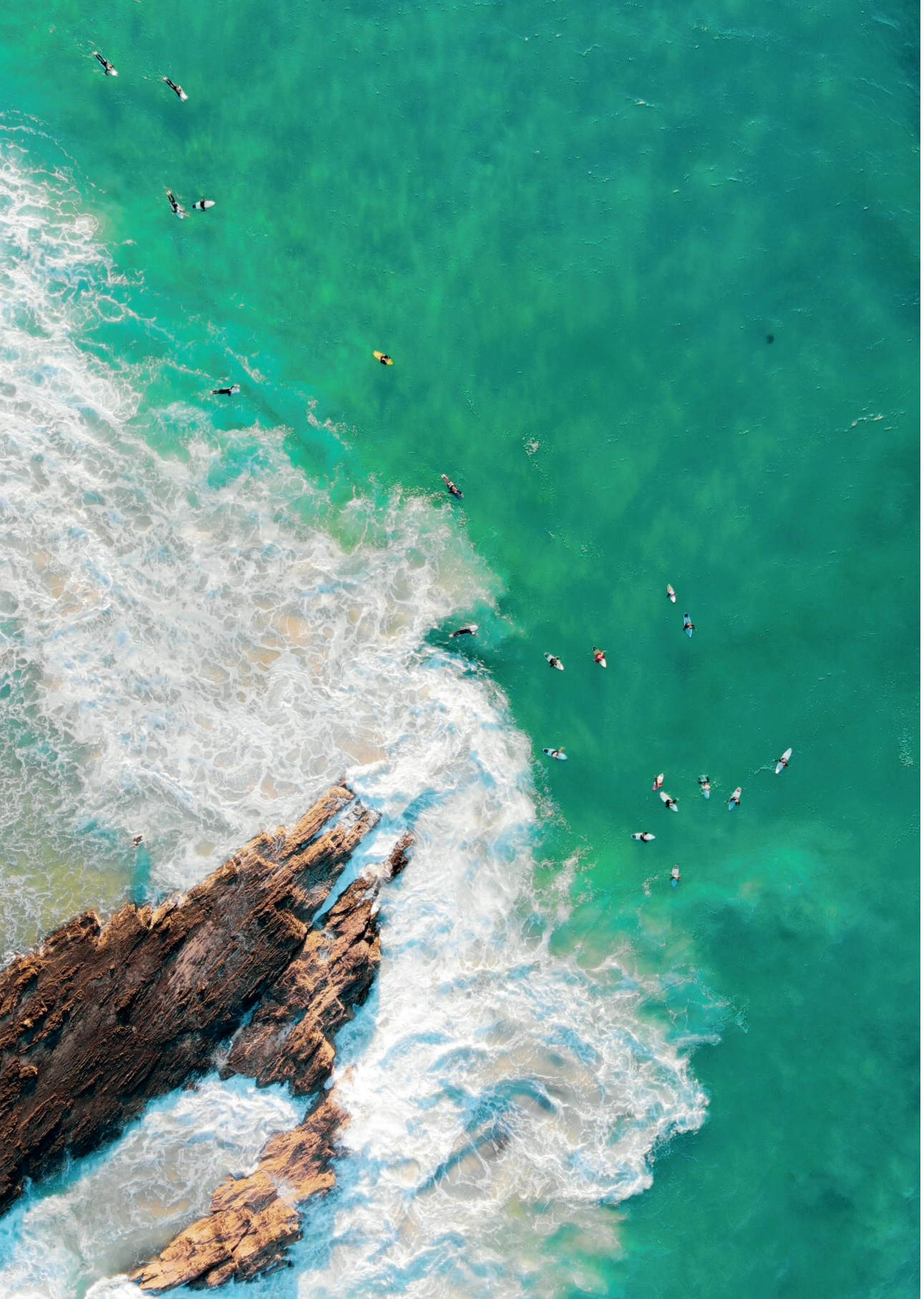
Research agenda
- More high quality, prospective evidence needed on appropriate surveillance intervals and risk categories
- Develop and validate non-invasive methods for risk assessment and neoplasia detection
- Develop ways of quantifying cumulative (mutagenic) inflammatory damage
- Histopathologic score to be used for quantifying inflammation
- Differentiation between dysplasia types using biomolecular and genetic markers. Harmonize variation in terminology for dysplastic lesions.
- Develop strategies for reducing unnecessary examinations
- Determine if HDWLE is truly comparable to CE surveillance
- Assess the predictive value of single or repeated negative (chromo)endoscopies
- International collaboration and prospective registration of IBD surveillance population for research purposes

Table 4. Comparison of the most-used guidelines

Society	Start surveillance	Risk stratification	Surveillance interval
ECCO (2017)	8 years after onset of symptoms	Stricture or dysplasia within past 5 years, PSC, extensive colitis with severe active inflammation	1 year
		Extensive colitis with mild to moderate active inflammation, post-inflammatory polyps, or first-degree relative with CRC > 50 years	2-3 years
		No intermediate- or high-risk features	5 years
AGA (2010)	8 years after disease onset	Active inflammation, stricture, post-inflammatory polyps, history of dysplasia, first-degree relative with CRC, PSC	1 year
		After 2 negative examinations	1-3 years
ACG (2010)	8-10 years after disease onset	No stratification	1-2years
BSG (2010)	10 years after onset of symptoms	Moderate or severe active inflammation on the previous surveillance examination, stricture or dysplasia within past 5 years, PSC, first-degree relative with CRC <50 years	1 year
		Mild active inflammation on the previous surveillance examination, post-inflammatory polyps, first-degree relative with CRC >50 years	3 years
		No active inflammation on the previous surveillance procedure, left-sided colitis or CD colitis affecting >50% surface area of the colon	5 years

REFERENCES

1. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
2. Farraye, F. A. *et al.* AGA medical position statement on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* **138,** 738–45 (2010).
3. Laine, L. *et al.* SCENIC International Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease. *Gastroenterology* **148,** 639–651.e28 (2015).
4. Cairns, S. R. *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* **59,** 666–89 (2010).
5. Magro, F. *et al.* Third European Evidence-Based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J. Crohn's Colitis* (2017). doi:10.1093/ecco-jcc/jjx008
6. Lutgens, M. W. M. D. *et al.* Colonoscopic surveillance improves survival after colorectal cancer diagnosis in inflammatory bowel disease. *Br. J. Cancer* **101,** 1671–5 (2009).
7. Iannone, A. *et al.* Chromoendoscopy for Surveillance in Ulcerative Colitis and Crohn's Disease: A Systematic Review of Randomized Trials. *Clin. Gastroenterol. Hepatol.* (2016). doi:10.1016/j.cgh.2016.11.021
8. Carballal, S. *et al.* Real-life chromoendoscopy for neoplasia detection and characterisation in long-standing IBD. *Gut* gutjnl-2016-312332 (2016). doi:10.1136/gutjnl-2016-312332
9. Lutgens, M. W. M. D. *et al.* High frequency of early colorectal cancer in inflammatory bowel disease. *Gut* **57,** 1246–1251 (2008).
10. Bisschops, R. *et al.* Chromoendoscopy versus narrow band imaging in UC: a prospective randomised controlled trial. *Gut* gutjnl-2016-313213 (2017). doi:10.1136/gutjnl-2016-313213
11. Mooiweer, E. *et al.* Chromoendoscopy for Surveillance in Inflammatory Bowel Disease Does Not Increase Neoplasia Detection Compared With Conventional Colonoscopy With Random Biopsies: Results From a Large Retrospective Study. *Am. J. Gastroenterol.* **110,** 1014–21 (2015).
12. Moussata, D. *et al.* Are random biopsies still useful for the detection of neoplasia in patients with IBD undergoing surveillance colonoscopy with chromoendoscopy? *Gut* gutjnl-2016-311892 (2017). doi:10.1136/GUTJNL-2016-311892
13. Watanabe, T. *et al.* Comparison of Targeted vs Random Biopsies for Surveillance of Ulcerative Colitis-associated Colorectal Cancer. *Gastroenterology* (2016). doi:10.1053/j.gastro.2016.08.002
14. Mooiweer, E. *et al.* Incidence of Interval Colorectal Cancer Among Inflammatory Bowel Disease Patients Undergoing Regular Colonoscopic Surveillance. *Clin. Gastroenterol. Hepatol.* **13,** 1656–1661 (2015).
15. Mooiweer, E., Fidder, H. H., Siersema, P. D., Laheij, R. J. F. & Oldenburg, B. Fecal Calprotectin Testing Can Identify Ineffective Colorectal Cancer Surveillance Procedures in Patients with Longstanding Colitis. *Inflamm. Bowel Dis.* **20,** 1 (2014).



CHAPTER 10

Circulating microRNAs for the detection colitis-associated neoplasia fail to validate in patients undergoing endoscopic surveillance

Manuscript in preparation

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ABSTRACT

Introduction: Patients with inflammatory bowel disease (IBD) have an increased risk of developing colorectal cancer. Currently, endoscopic surveillance is advocated in IBD patients to detect premalignant lesions or colorectal cancer (CRC) at an early stage. Colonoscopy is an invasive and relatively expensive procedure that imposes a burden on both the healthcare system and patients. The search for new, non-invasive markers capable of identifying patients at risk for neoplasia development and malignant transformation is therefore of great importance. In the field of biomarker discovery, microRNAs have received a great deal of attention over the past few years. These small, non-coding, 18-22 nucleotide RNAs have been implicated in the etiology of cancer and a number of chronic diseases, including IBD. The aim of this study was to identify a plasma-based panel of microRNAs capable of reliably predicting the presence of colorectal neoplasia in IBD patients.

Methods: Patients undergoing endoscopic surveillance were enrolled in a prospective cohort. Inclusion criteria were a confirmed diagnosis of ulcerative colitis, Crohn's colitis or indeterminate colitis, age 18-17 years, a disease-duration of more than 8 years and involvement of at least 30 percent of the colon. Before each surveillance colonoscopy, plasma samples were collected and stored at -80˚C. RNA was extracted using the miRcury RNA Isolation kit for Biofluids (Exiqon).

Using an Openarray platform (LifeTechnologies), the levels of 758 miRNAs were evaluated in the plasma of 16 IBD-neoplasia patients and 16 IBD patients without neoplasia. As an additional comparison, these analyses were performed in 10 healthy (non-IBD) control samples. Levels of each microRNA were normalized to the levels of the exogenous spike-in ath-miR-159a. After selection of differentially expressed microRNAs and a technical technical validation step, miRNA specific single Taqman miRNA RealTime-quantitative PCR (RT-qPCR) assays were performed on an independent validation cohort.

Results: In total 42 patients were included in the discovery cohort (16 IBD dysplasia, 16 IBD controls, 10 healthy controls). Five miRNAs (mir-210, mir-345, mir-598, mir-1274A and mir-320B) were differentially expressed in the plasma of IBD-neoplasia patients. However, in the independent validation cohort (20 IBD dysplasia, 20 IBD controls) these results could not be replicated by single RT-qPCR.

Conclusion: In this explorative study, five miRNAs were found to be increased in the plasma of patients with IBD and colorectal neoplasia. However, we were unable to reproduce these findings in a separate validation study. Although miRNAs have the potential to serve as diagnostic biomarkers, we were unable to reliably distinguish IBD dysplasia patients from IBD controls.

INTRODUCTION

Patients with long-standing colonic inflammatory bowel disease (IBD) have an increased risk of developing colorectal cancer. It is generally accepted that the risk of IBD-associated colorectal cancer (CRC) exceeds that of the background population by a factor of 2[1], and that this effect is comparable for ulcerative colitis and Crohn's colitis.[2] It is assumed that the increased risk is caused by exposure of the colonic mucosa to chronic inflammation, which leads to dysplasia and ultimately to colorectal cancer.[3] Currently, endoscopic surveillance is advocated in IBD patients to detect premalignant lesions or CRC at an early stage. However, the level of evidence for the effectiveness of surveillance is low as no prospective randomized controlled trials have been performed, although improved survival for patients undergoing surveillance was found in several case control studies.[4,5]

Endoscopic surveillance in colitis is a challenging procedure that relies heavily on the ability to accurately detect pre-neoplastic lesions with high positive and negative predictive values for progression to CRC. Colonoscopy is an invasive and relatively expensive procedure that imposes a burden on both the healthcare system and patients. The search for new, non-invasive markers capable of identifying patients at risk for neoplasia development and malignant transformation is therefore of great importance. However, the need for a robust biomarker in this setting is currently unmet.

In the field of biomarker discovery, microRNAs (miRNAs) have received a great deal of attention over the past few years. These small, non-coding, 18-22 nucleotide RNAs have been implicated in the etiology of cancer and a number of chronic diseases, including IBD.[6–8] A multitude of studies has reported that miRNAs play a key role in regulating cell differentiation, proliferation and apoptosis at the post-transcriptional level.[9,10]

The significant differences in miRNA expression profiles between CRC tissue and normal tissue and their stability in circulation suggest that these parameters might serve as blood-based biomarkers for the detection of CRC.[11,12] The aim of the current study was therefore to explore the potential of microRNAs to reliably predict the

presence of neoplasia in IBD patients at risk, employing a plasma-based panel (discovery cohort). Furthermore, we aimed to validate our findings in an independent subgroup of patients (validation cohort).

METHODS

Patient selection

Patients between18 and 70 years of age with a confirmed diagnosis of ulcerative colitis (UC), Crohn's disease (CD) or indeterminate colitis (IBD-U) were enrolled in a prospective cohort. All patients had an indication for endoscopic surveillance according to the current guidelines[13,14], i.e. have a disease-duration of more than 8 years and involvement of at least 30 percent of the colon. Eligible patients were asked to participate in this study by their treating physician.

Following informed consent, patients underwent state-of-the-art surveillance colonoscopies, performed by employing chromoendoscopy with a high definition colonoscope. During colonoscopy, all abnormal lesions suspected of dysplasia or carcinoma were biopsied or directly removed if possible. Tissue specimens were reviewed by two expert gastrointestinal pathologists for the presence of low or high-grade dysplasia or colorectal cancer. Before each surveillance colonoscopy, a blood sample of 20 ml was collected, plasma was isolated and stored at -80˚C. All included patients were asked to fill out a questionnaire, with a special focus on potential risk factors for CRC development, medication use, smoking and diet. Patients received follow-up endoscopic procedures in accordance with the European guidelines for surveillance in IBD, performed at either 5, 3 or 1 year intervals.[13] The same blood sampling protocol described for the initial endoscopy were used for follow-up visits.

From the full prospective cohort, patients were selected for the initial discovery study. Sixteen patients were selected in whom a large dysplastic lesion or cancer was detected during endoscopic surveillance. All lesions occurred in an area previously affected by colitis and were morphologically classified as colitis-associated. A age- and sex-matched control group of equal size (n=16) was selected from patients in whom there was no history of dysplasia or CRC and in whom two consecutive

prospective surveillance colonoscopies were negative for dysplasia. As an additional comparison group, 10 healthy (non-IBD) control subjects were included in the screening study. (Figure 1)

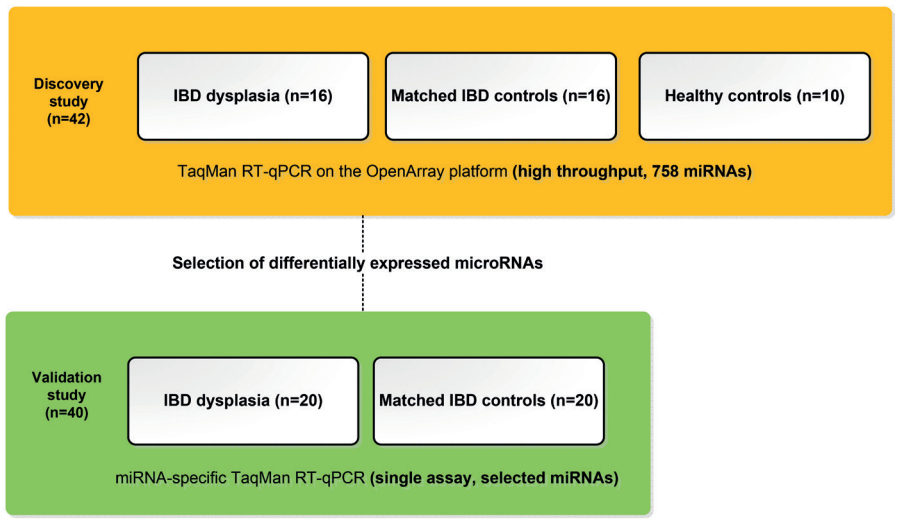


Figure 1. Study design

miRNA analyses

Blood samples were collected in Vacutainer® K2EDTA tubes (BD Vacutainer) and plasma was isolated after centrifugation at 1500 g for 10 minutes. Immediately after plasma isolation, aliquots were stored at -80˚C.

RNA was extracted using the miRcury RNA isolation kit for Biofluids (Exiqon), according to the manufacturer's protocol. At this step, a non-human exogenous spike-in miRNA (synthetic Arabidopsis thaliana ath-miR-159a) was added to each sample to allow for data normalization later on. In addition, the exogenous spike-in miRNA was monitored to observe for technical variability during RNA isolation.

For the discovery study, miRNA profiling was performed by using TaqMan RealTime-quantitative PCR (RT-qPCR) on the OpenArray platform (Life Technologies), a method that allows for simultaneous analysis of 758 miRNAs (split into two pools). 2.5ul of

isolated plasma RNA was reverse-transcribed using the miRNA multiplex primer pools. After data analysis, miRNAs sufficiently up- or downregulated between the IBD dysplasia and IBD control groups were selected and analyzed in a separate cohort of patients.

Technical validation

Prior to the assessment of the selected microRNAs in a separate cohort, we first analyzed the correlation between platforms, i.e. OpenArray and single assay RT-qPCR. Both platforms employ PCR, yet the OpenArray platform used in the profiling study is a high-throughput platform. For each of the samples used in the profiling study, we performed a technical replication using single assay RT-qPCR. The results were plotted and the correlation between these two assays were analyzed for each selected microRNA.

Biological validation

After selection of differentially expressed miRNAs and following technical replication as single assay, we sought to quantify these miRNAs in the validation cohort. The quantification of miRNA expression in plasma was performed by single Taqman RT-qPCR assays (Life Technologies). In short, 2.5 ml of serum RNA was reverse-transcribed using the specific microRNA assays (ath-miR159a; hsa-miR-210, hsa-miR-345, hsa-miR-598, hsa-miR-1274A, hsa-miR-320B) and measured with the specific TaqMan assay on the QuantStudio 12k flex System, in the presence of the TaqMan Fast Advanced Master mix (Life Technologies), following the manufacturer's instructions.

Statistical analysis

The Relative Quantification method of Expression Suite Software (ThermoFisher) was applied to analyze the data by using the comparative threshold cycle method (Crt). Low expressed miRNAs were excluded from the analysis: the Crt threshold was set at 27 and only miRNAs with high amplification quality (>1.24) were taken into

consideration. The expression of each miRNA was calculated after normalization on the levels of the exogenous spike-in ath-miR-159a and expressed as Fold Change (FC) as compared to the control samples.

GraphPad Prism 7.04 (San Diego, CA) and IBM SPSS 25 (Armonk, NY) were used for the subsequent analyses in the study. Kruskal-Wallis, Mann-Whitney U test and Wilcoxon signed rank test were applied to compare data that were not normally distributed as indicated. Spearman's rho was used to assess correlations. Differences were considered significant at $p < .05$ (uncorrected p-value).

Ethical considerations

This study was approved by the Medical Ethical Committee of the University Medical Center Utrecht. Written consent was be obtained from all patients prior to obtaining plasma samples.

Table 1. Patient characteristics for the discovery and validation cohorts

	Discovery cohort (n=42)				Validation cohort (n=40)		
	Healthy controls (n=10)	**IBD controls (n=16)**	**IBD dysplasia (n=16)**	**P-value**	**IBD Controls (n=20)**	**IBD dysplasia (n=20)**	**P-value**
Male sex	6	11	13	0.41	9	9	1.0
Age, y (mean, SD)	48	52.2 (11.6)	55.8 (15.7)	0.90	54.9 (10.9)	55.4 (11.0)	0.87
Age of IBD diagnosis, y (mean, SD)		29.4 (12.4)	30.0 (13.4)	0.47	34.8 (14.4)	33.6 (11.8)	0.78
IBD							
- UC		5	5		9	12	
- CD		9	9	1.0	11	8	0.34
- IBD-U		2	2		-	-	
PSC	-	2	2	1.0	0	1	0.31
Dysplasia grade							
- LGD			12			18	
- HGD	-	-	1		-	0	
- CRC			3			2	

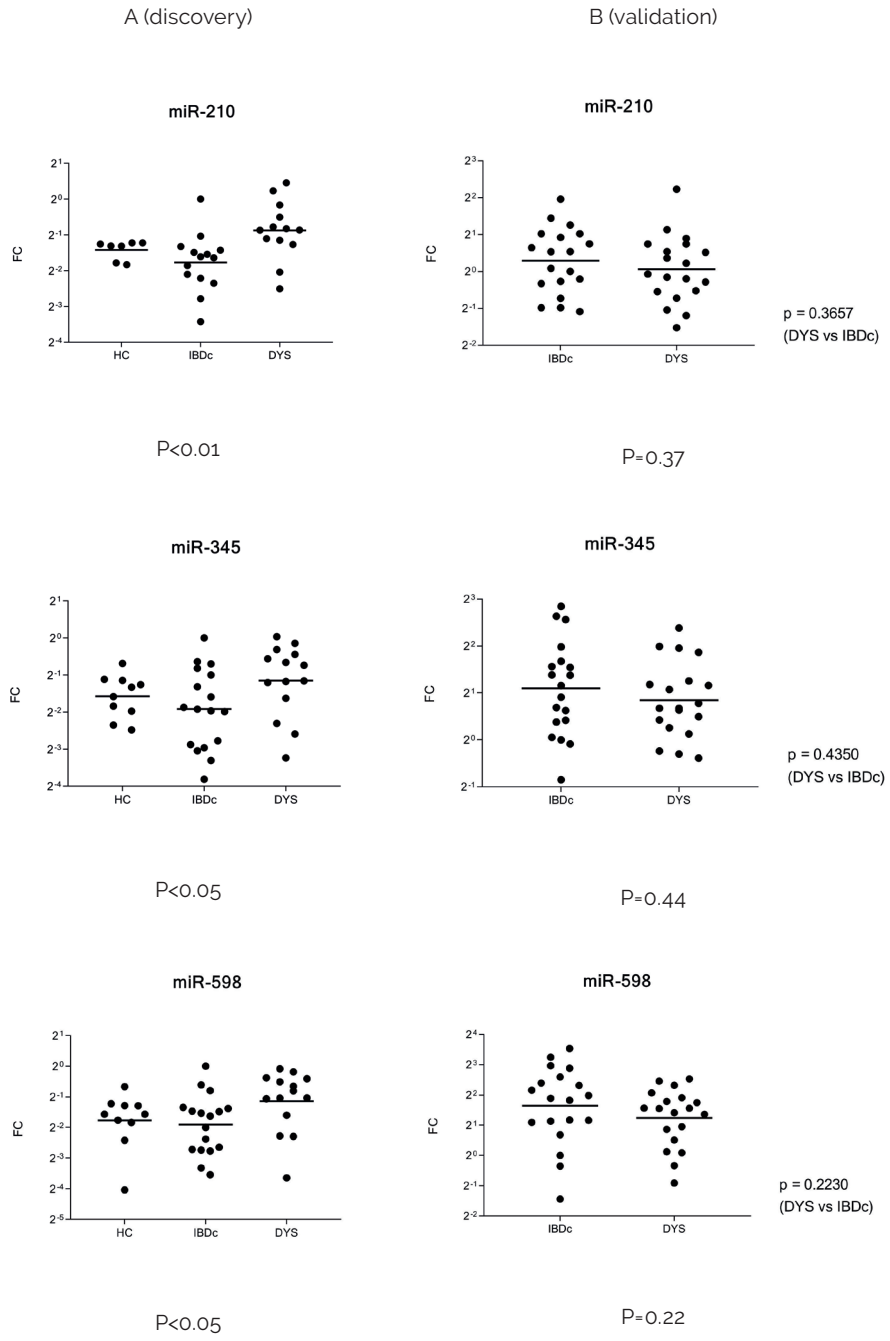
RESULTS

In total 42 patients were included in the screening study (16 IBD dysplasia, 16 IBD controls, 10 healthy controls). Age, sex and IBD subtype were equally distributed between cases and controls in both the discovery and validation cohorts. (Table 1)

The expression patterns of 758 miRNAs were analyzed in all three groups in the discovery study. One sample showed a low detection based on Crt of the spike-in miRNA and was removed from subsequent analysis. Five miRNAs were differentially expressed between IBD controls and dysplasia patients, all showing an increased expression in the latter group. (Figure 2A)

Table 2. Differentially expressed miRNAs in plasma of IBD dysplasia patients (comparison group: IBD controls)

miRNA	Fold change	P-Value
hsa-miR-210	1.862	0.008
hsa-miR-345	1.700	0.048
hsa-miR-598	1.694	0.042
hsa-miR-1274A	2.760	0.006
hsa-miR-320B	1.865	0.036



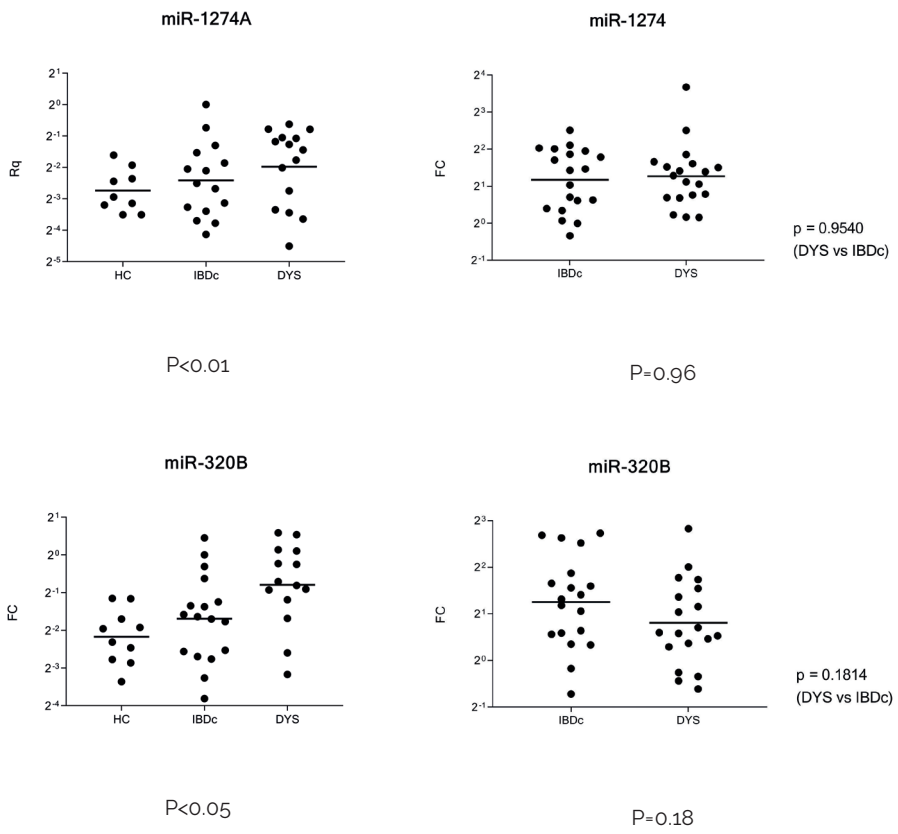


Figure 2. FC expression graphs for discovery (A; OpenArray) and validation (B; quantitative RT-qPCR) cohorts. (HC=healthy controls, IBDc=IBD controls without neoplasia, DYS=IBD patients with colitis associated neoplasia, FC=fold change) Expression values are displayed as Fold Change (FC) compared to the IBD control group. P-values are given for IBDc vs DYS.

Technical validation

Results of the high-throughput (Openarray) study versus the results measured by single assay RT-PCR in the same sample are displayed in supplementary figure 1, with correlation coefficients ranging from 0.49 to 0.89 . Since the correlations ranged from reasonable to good, all of the preselected miRNAs were studied further in the validation cohort.

Biological validation

In the validation cohort, we performed single RT-qPCR assays on 40 independent plasma samples (20 cases, 20 IBD controls) for hsa-miR-210, hsa-miR-345, hsa-miR-598, hsa-miR-1274A, hsa-miR-320B. Two samples (1 in each subgroup) were omitted from the analysis due to quality issues during RNA isolation. In contrast to the discovery cohort, none of the selected microRNAs were significantly differentially expressed in the validation cohort. (Figure 2B)

DISCUSSION

Patients with longstanding colitis are at an increased risk of developing CRC. Although some clinical parameters help to further quantify this risk, there is a lack of biomarkers that can improve the surveillance and identification of patients at high risk of developing CRC.

The aim of this study was to find new predictors of CRC development in IBD patients, by studying differential expression patterns of 758 miRNAs in a cohort of IBD patients undergoing active endoscopic surveillance. In the discovery study, employing high-throughput PCR, 5 miRNAs (hsa-miR-210, hsa-miR-345, hsa-miR-598, hsa-miR-1274A, hsa-miR-320B) were found to be increased in the plasma of IBD patients with significant dysplasia. However, in the subsequent validation phase, none of these miRNAs were significantly increased and thus we were unable to confirm the initial results.

In attempting to explain the inability to validate our findings, a number of aspects about this study should be considered. Firstly, there may be differences in patient or tumor characteristics that could have contributed to differences in miRNA expression levels. Circulating miRNAs have been associated with a large number of clinical factors as well as inflammatory conditions, including IBD.[8,15–17] For oncogenic miRNAs, the causal links to cancerous tissue and an inflammatory response may be intertwined.[18] Attempting to quantify cancer-specific miRNAs in IBD patients may prove especially challenging, given the variable background of inflammation in these patients.

We attempted to minimize the role of other clinical factors in this study through several ways. Firstly, the base population of our cohort had an indication for surveillance, i.e. all patients had significant and longstanding colitis of at least 30% of the colon. In this way, we kept the study population as close to the target population as possible. In addition, patients in the case and control groups were matched based on age and sex.

It should be highlighted that a majority of the lesions selected for this study were in a dysplastic (non-invasive, non-metastatic) stage. We took care in selecting lesions of significant size and (colitis-associated) morphology, since detection and removal of these lesions would improve survival and warrant the accompanying screening method as effective. However, circulating miRNAs may only become significantly elevated (or downregulated) once carcinogenesis has reached a stage beyond dysplasia. If indeed sensitivity of miRNA profiling becomes adequate once progression to CRC has taken place, the clinical applicability of miRNAs in this specific screening setting should be questioned.

Another possible explanation for the failure to validate our findings could be related to technical factors. However, the protocols and platforms used in our study have been used successfully in the past. The high-throughput platform we employed in this study has shown to be reliable for the detection of circulating miRNAs and has repeatedly been successfully applied in other studies.[19,20] Furthermore, storage and extraction techniques (which can significantly affect RNA quality) were similar for both cohorts. Since this was a prospective cohort study, we were able to ensure that all plasma samples were taken before a lesion was endoscopically or surgically removed. In addition, we incorporated a technical replication step, which showed good correlation between the OpenArray (high-throughput) and single assay RT-PCR platforms. Given that the findings correlated well, biological variations are more likely to explain the inability to replicate these findings than technical factors.

Thirdly, a possible factor explaining the inability to replicate our findings could be that the initial subset of miRNAs was wrongfully selected and the observed increases in plasma of IBD dysplasia patients should be considered false positive results. More strict selection (for example by setting a higher threshold for fold change and by

employing stricter correction for multiple testing) in the discovery cohort would lower the chances of false positive results. However, since we planned a validation step, we chose to be more lenient so as not to risk false negative results by discarding potentially useful miRNAs.

In recent times, a large number of studies have proposed differentially expressed miRNAs as aids in the diagnosis of various cancers and in determining prognosis. Circulating miRNAs have been studied as biomarkers for colorectal cancer in the general population, with consistent upregulation found for miR-21, miR-29a and miR92a.[21–24] One previous study assessed the potential role of microRNAs as blood based biomarkers in the setting of IBD surveillance and found miR-375 to be significantly upregulated in the colitis-associated CRC subgroup.[25] Since no full report is as of yet published, it is unclear whether the investigators also used an external validation cohort to confirm this specific finding. The relative increase of miR-375 was not replicated in our study.

In addition to studies on circulating miRNAs, a significant number studies has investigated the expression patterns of miRNAs in colitis-associated cancer tissues, both in mice and humans.[26–33] A recent study by Toiyama et al. looked at methylation of specific miRNAs in rectal biopsies of UC patients and found the methylation status to correlate to several clinical factors, including disease duration.[34] Importantly, they found that the amount of methylation was significantly higher in patients with dysplasia or cancer compared to UC patients without neoplasia, which was validated in an independent cohort of patients.

Additionally, fecal miRNAs have reported to be differentially expressed in patients with sporadic CRC.[35,36] Research on fecal miRNAs has also provided evidence that these may correlate with disease activity in IBD patients.[37] Whether miRNAs detected in stool samples may reliably predict disease states, remains to be seen.

While the field of microRNA discovery is promising, caution remains warranted in linking miRNAs to specific diseases, for they are generally not disease or organ specific.[38]

In summary, our study did not show reproducible diagnostic properties for circulating microRNAs in plasma of IBD patients with colorectal dysplasia. For a biomarker to be actively used in clinical practice, the associations should be robust and replicated in independent cohorts. We deliberately chose to investigate circulating miRNAs, since tissue based microRNAs would still necessitate invasive endoscopic procedures including biopsy and/or polypectomy. Although several study related factors, both technical and clinical, may explain the negative findings, it could also be argued that a clinically relevant circulating microRNA in the detection of colitis-associated dysplasia simply does not exist.

Strengths of our study include the large, unbiased selection of miRNAs used in the discovery cohort and the prospective design. Our study also has limitations. The number of cases in both the discovery and validation cohorts is relatively small, with few cases of CRC. Secondly, the criteria for selection of miRNAs were relatively lenient, increasing the risk of false positive findings.

CONCLUSIONS

Predicting the risk of colitis-associated CRC remains a challenge and the current endoscopic surveillance programs place a burden on patients. We sought to explore the potential role of circulating miRNAs in the detection of colitis associated neoplasia. Although several plasma based miRNAs were increased in the discovery cohort, these same associations were not detected in a separate cohort of patients. In summary, expression patterns of miRNAs in plasma did not reliably distinguish IBD dysplasia patients from IBD controls in this study.

REFERENCES

1. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
2. Ekbom, A., Helmick, C., Zack, M. & Adami, H. O. Increased risk of large-bowel cancer in Crohn's disease with colonic involvement. *Lancet* **336,** 357–9 (1990).
3. Itzkowitz, S. H. & Yio, X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287,** G7-17 (2004).
4. Lutgens, M. W. M. D. *et al.* Colonoscopic surveillance improves survival after colorectal cancer diagnosis in inflammatory bowel disease. *Br. J. Cancer* **101,** 1671–5 (2009).
5. Karlén, P. *et al.* Is colonoscopic surveillance reducing colorectal cancer mortality in ulcerative colitis? A population based case control study. *Gut* **42,** 711–4 (1998).
6. Kanaan, Z. *et al.* Plasma miR-21: a potential diagnostic marker of colorectal cancer. *Ann. Surg.* **256,** 544–51 (2012).
7. He, L. *et al.* A microRNA component of the p53 tumour suppressor network. *Nature* **447,** 1130–4 (2007).
8. Kalla, R. *et al.* MicroRNAs: new players in IBD. *Gut* **64,** 504–17 (2015).
9. Calin, G. A. & Croce, C. M. MicroRNA signatures in human cancers. *Nat. Rev. Cancer* **6,** 857–66 (2006).
10. Chen, X. *et al.* Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res.* **18,** 997–1006 (2008).
11. Visone, R. & Croce, C. M. MiRNAs and cancer. *Am. J. Pathol.* **174,** 1131–8 (2009).
12. Mazeh, H. *et al.* The Diagnostic and Prognostic Role of microRNA in Colorectal Cancer - a Comprehensive review. *J. Cancer* **4,** 281–95 (2013).
13. Magro, F. *et al.* Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J. Crohn's Colitis* **11,** 649–670 (2017).
14. Cairns, S. R. *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* **59,** 666–89 (2010).
15. Ranjha, R., Aggarwal, S., Bopanna, S., Ahuja, V. & Paul, J. Site-Specific MicroRNA Expression May Lead to Different Subtypes in Ulcerative Colitis. *PLoS One* **10,** e0142869 (2015).
16. Schaefer, J. S. *et al.* MicroRNA signatures differentiate Crohn's disease from ulcerative colitis. *BMC Immunol.* **16,** 5 (2015).

17. Lewis, A. *et al.* Low Serum Levels of MicroRNA-19 Are Associated with a Stricturing Crohn's Disease Phenotype. *Inflamm. Bowel Dis.* **21,** 1926–34 (2015).

18. Iliopoulos, D. MicroRNA Circuits Regulate the Cancer-Inflammation Link. *Sci. Signal.* **7,** pe8-pe8 (2014).

19. Farr, R. J. *et al.* A comparative analysis of high-throughput platforms for validation of a circulating microRNA signature in diabetic retinopathy. *Sci. Rep.* **5,** 10375 (2015).

20. Chouri, E. *et al.* Serum microRNA screening and functional studies reveal miR-483-5p as a potential driver of fibrosis in systemic sclerosis. *J. Autoimmun.* **89,** 162–170 (2018).

21. Shigeyasu, K., Toden, S., Zumwalt, T. J., Okugawa, Y. & Goel, A. Emerging Role of MicroRNAs as Liquid Biopsy Biomarkers in Gastrointestinal Cancers. *Clin. Cancer Res.* **23,** 2391–2399 (2017).

22. Kanaan, Z. *et al.* A plasma microRNA panel for detection of colorectal adenomas: a step toward more precise screening for colorectal cancer. *Ann. Surg.* **258,** 400–8 (2013).

23. Wang, Q. *et al.* Plasma miR-601 and miR-760 are novel biomarkers for the early detection of colorectal cancer. *PLoS One* **7,** e44398 (2012).

24. Carter, J. V. *et al.* A Highly Predictive Model for Diagnosis of Colorectal Neoplasms Using Plasma MicroRNA. *Ann. Surg.* **264,** 575–584 (2016).

25. Patel, M., Verma, A., Aslam, I., Pringle, H. & Singh, B. Novel plasma microRNA biomarkers for the identification of colitis-associated carcinoma. *Lancet (London, England)* **385 Suppl,** S78 (2015).

26. Shi, C. *et al.* Novel evidence for an oncogenic role of microRNA-21 in colitis-associated colorectal cancer. *Gut* **65,** 1470–81 (2016).

27. He, C. *et al.* MicroRNA 301A Promotes Intestinal Inflammation and Colitis-Associated Cancer Development by Inhibiting BTG1. *Gastroenterology* **0,** (2017).

28. Polytarchou, C. *et al.* MicroRNA214 Is Associated With Progression of Ulcerative Colitis, and Inhibition Reduces Development of Colitis and Colitis-Associated Cancer in Mice. *Gastroenterology* **149,** 981–992.e11 (2015).

29. Mo, J.-S., Han, S.-H., Yun, K.-J. & Chae, S.-C. MicroRNA 429 regulates the expression of CHMP5 in the inflammatory colitis and colorectal cancer cells. *Inflamm. Res.* **67,** 985–996 (2018).

30. Feng, Y. *et al.* MicroRNA-449a is a potential predictor of colitis-associated colorectal cancer progression. *Oncol. Rep.* **40,** 1684–1694 (2018).

31. Pekow, J. *et al.* miR-193a-3p is a Key Tumor Suppressor in Ulcerative Colitis–Associated Colon Cancer and Promotes Carcinogenesis through Upregulation of IL17RD. *Clin. Cancer Res.* **23,** 5281–5291 (2017).

32. Lewis, A. *et al.* The miR-200 family is increased in dysplastic lesions in ulcerative colitis patients. *PLoS One* **12,** e0173664 (2017).

33. Pekow, J. *et al.* miR-4728-3p Functions as a Tumor Suppressor in Ulcerative Colitis-associated Colorectal Neoplasia Through Regulation of Focal Adhesion Signaling. *Inflamm. Bowel Dis.* (2017).

34. Toiyama, Y. *et al.* A Panel of Methylated MicroRNA Biomarkers for Identifying High-Risk Patients with Ulcerative Colitis-associated Colorectal Cancer. *Gastroenterology* (2017).

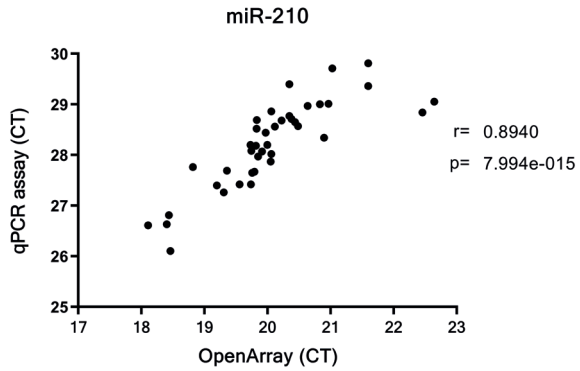
35. Ahmed, F. E. *et al.* Diagnostic microRNA markers for screening sporadic human colon cancer and active ulcerative colitis in stool and tissue. *Cancer Genomics Proteomics* **6,** 281–95

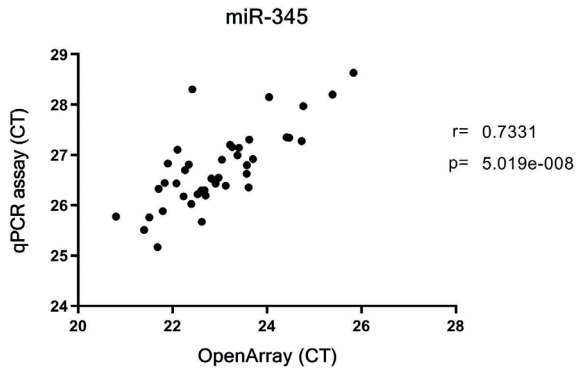
36. Link, A. *et al.* Fecal MicroRNAs as novel biomarkers for colon cancer screening. *Cancer Epidemiol. Biomarkers Prev.* **19,** 1766–74 (2010).

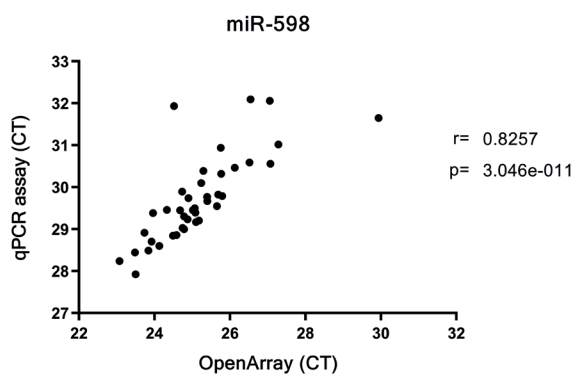
37. Schönauen, K. *et al.* Circulating and Fecal microRNAs as Biomarkers for Inflammatory Bowel Diseases. *Inflamm. Bowel Dis.* **24,** 1547–1557 (2018).

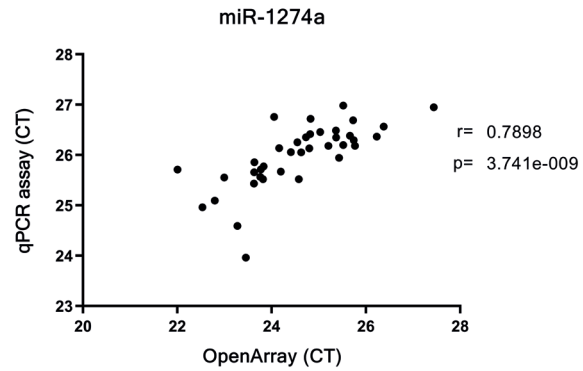
38. Pritchard, C. C. *et al.* Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. *Cancer Prev. Res. (Phila).* **5,** 492–7 (2012).

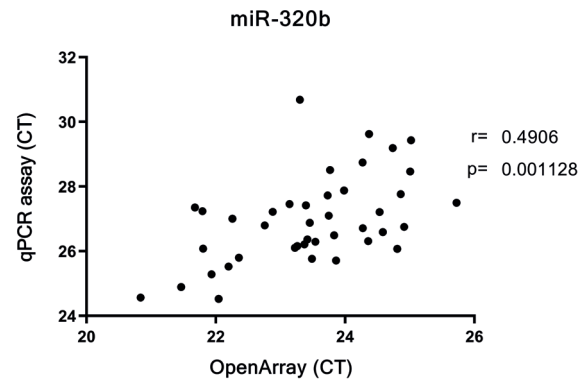
SUPPLEMENTARY MATERIALS











Supplementary figure 1. Technical validation

CHAPTER 11

Summary

The first section of this thesis was directed at providing an adequate and up-to-date definition of the population at risk, exploring risk factors and determining the risk of progression from low-grade dysplasia to advanced neoplasia i.e., high grade dysplasia or cancer. Moreover, we estimated the incidence rates and neoplasia-free intervals after negative colonoscopies.

Chapter 2 reports on a retrospective study in which we assessed the risk of advanced colorectal neoplasm in a cohort of patients enrolled in endoscopic surveillance programs in the Netherlands and the United States (US). For a total of 1911 patients, we included data on clinical and demographic factors, as well as longitudinal data on each surveillance colonoscopy performed from 2000 through 2015.

The findings in this study point towards an increased risk for patients with concomitant PSC: these patients had an approximately twofold increased risk of developing advanced neoplasia (HGD or CRC) as compared with patients with non-PSC IBD. This increase in risk was independent from other risk factors such as age and active inflammation. Among PSC-IBD patients, there was also a higher rate of developing advanced neoplasia after LGD had been detected. A third finding was that LGD was more often endoscopically invisible in patients with PSC-IBD compared with patients with non-PSC IBD. The findings in this study support the more intensive surveillance strategy that already has been implemented in patients with the PSC-IBD 'phenotype'.

In **Chapter 3**, we explored the risk of advanced neoplasia following the detection of LGD, with a focus on differences in endoscopic quality and adjuncts that have been used over the last decade. In particular, we distinguished lesions detected by white light endoscopy from lesions detected using chromoendoscopy.

In a Dutch multicenter cohort, we were able to identify 159 patients who underwent endoscopic follow-up after the detection of (uni- or multifocal) LGD. The risk of advanced neoplasia during follow-up was small with an overall incidence rate of advanced neoplasia of 1.34 per 100 patient-years with a median follow-up of 4.7 years. From the moment of finding the 'index' LGD lesion, the median time to advanced

neoplasia was 3.3 years. As expected, we did not identify a difference in risk when comparing LGD lesions detected with either chromoendoscopy or WLE.

In **Chapter 4** we focused on the risk of advanced neoplasia in patients undergoing surveillance in whom the risk of progression was considered low, notably in whom two separate colonoscopies were 'negative' for dysplasia and other high risk-features.

To do this, we constructed a multicenter, multinational database of patients with long-standing IBD colitis without high-risk features from three countries; The Netherlands, the US and Canada. A 'negative' surveillance colonoscopy was predefined as a technically adequate procedure during which no post-inflammatory polyps ('pseudopolyps'), no strictures, no endoscopic disease activity and no dysplastic lesions were detected. In contrast, a 'positive' colonoscopy was a technically adequate procedure that included at least one of these criteria.

Of the 775 patients with long-standing IBD colitis, 340 had at least 1 negative colonoscopy. Over a follow-up period of 6.1 years after the index procedure, we found that no advanced neoplasia occurred in those with consecutive negative surveillance colonoscopies, compared with an incidence rate of 0.29 to 0.76/100 patient-years (P=0.02) in those having one or more positive colonoscopies. These findings suggest that longer surveillance intervals in this selected population may be safe.

Chapter 5 zooms in on a subgroup of IBD patients that is often overlooked when considering the risk of future colonic neoplasia. In this study, we used the National Pathology database (PALGA) to identify IBD patients with a diverted rectum after colonic surgery. We then collected specific clinical and endoscopic data to assess the risk of advanced neoplasia in the rectal stump, as well as other long-term rectal stump related complications.

We included 250 patients in whom the rectal stump was left in situ for more than 12 months. During follow-up, the incidence rates for cancer plus dysplasia and cancer alone were 3.9 and 8.5 per 1000 patient-years of follow-up, respectively.

In the second part of this study, we set out to identify differences in patient-reported outcome measures (PROMs) associated with the excision of the rectal stump, by

using a questionnaire. Among 165 respondents (patients with either a rectal stump in situ or who had undergone a proctectomy), no significant differences in quality of life between patients with and patients without a rectal stump were found. However, patients who had undergone a proctectomy reported significantly more sexual and urinary symptoms than patients with a rectal stump in situ.

The first section of this thesis included a range of studies exploring risk estimates and risk factors, whereas section 2 was aimed at exploring the technical side of endoscopic surveillance and new strategies for improving its effectiveness.

Chapter 6 provides an overview of established surveillance strategies as advocated in guidelines as well as innovative endoscopic techniques. A major focus of this chapter is chromoendoscopy, a technique that involves the application of a dye to improve the delineation of mucosal abnormalities. We presented an overview of trials that were performed to assess dysplasia detection rates in white-light endoscopy and chromoendoscopy. The majority of trials showed superior dysplasia detection rates during chromoendoscopy compared to white light endoscopy, prompting the major guidelines to advocate this technique as the new gold standard.

We also explored the evidence on other image-enhanced endoscopy techniques within the context of IBD surveillance. First, narrow-band imaging (NBI), a technique based on narrowing the spectrum of light emitted from the endoscope, was evaluated. Due to a lack in improvement of dysplasia detection rates, current evidence does not support using this technique in IBD surveillance. Similarly, both FICE and i-scan (other digital chromoendoscopy techniques) as well as auto-fluorescence imaging (AFI) are not advocated in IBD surveillance, due to a lack of evidence in this patient group.

In **Chapter 7**, we evaluated a common recommendation in IBD surveillance guidelines, which states that biopsies should be taken from the mucosa surrounding dysplastic lesions. Thus far, no study had been performed on the actual dysplasia yield from biopsies of surrounding mucosa. In this study we identified 140 dysplastic lesions (in 71 patients) in which mucosal biopsies were collected from tissues adjacent to the lesion. The mean number of surrounding mucosa biopsies collected per lesion was

3.4 (range, 1–6) and dysplasia was detected in 7 biopsies surrounding 140 areas of dysplasia (5.0%). In addition, we studied the follow-up of these patients with dysplasia in mucosa surrounding lesions of low-grade dysplasia and found that post-resection surveillance did not reveal high-grade dysplasia or colorectal cancer in any of the cases. We concluded that these results cast doubt on the usefulness and cost-effectiveness of routinely taking biopsies of surrounding mucosa.

With **Chapter 8,** we aimed to study the feasibility and safety of endoscopic resection of large (≥ 20 mm) lesions in a colon with signs of inflammation. Previously, these patients were almost universally referred for surgery. However, most visible lesions encountered in IBD patients are well delineated and can be safely resected endoscopically, as long as the resection is complete.

We included 19 IBD patients, in whom lesions with a mean size of 30mm (range 20-100mm) were removed endoscopically. The procedures included both endoscopic mucosal resection (EMR, n=12) as well as endoscopic submucosal dissection (ESD, n=7). During follow-up, in 1 case recurrent LGD at the scar was observed. Two patients were found to have synchronous lesions (1 CRC, 1 sessile serrated adenoma (SSA)). One patient developed a metachronous lesion (>1y) with HGD and in three patients sporadic adenomas (LGD) were removed during follow-up.

In **Chapter 9**, we touched upon common encountered difficulties in performing endoscopic surveillance in IBD surveillance and discuss in detail a number of unresolved questions related to this subject.

We stress the importance of some key factors in the management of IBD patients eligible for surveillance, notably: enduring remission, optimal bowel preparation when performing surveillance colonoscopies, discussing findings of dysplasia in a multidisciplinary team and identifying high-risk groups.

Although guidelines provide an important framework for surveillance, new evidence has been published providing insights that have not yet made it into the guidelines. An important example is the fact that routinely taking random biopsies in all IBD surveillance patients is now considered ineffective. Moreover, there are regional

differences (notably European vs. North American guidelines) in risk stratification and surveillance intervals used in daily practice.

Chapter 10 reports a prospective study, aimed at identifying new, non-invasive, blood-based biomarkers with the potential to improve risk stratification of colitis-associated dysplasia. We specifically focused on a plasma-based panel of microRNAs capable of reliably predicting the presence of colorectal neoplasia in IBD patients. We included 42 patients in a screening study (16 IBD dysplasia, 16 IBD controls and 10 healthy controls) and used the Openarray platform (LifeTechnologies) to evaluate the levels of 758 miRNAs in plasma of the included patients.

Five miRNAs (mir-210, mir-345, mir-598, mir-1274A and mir-320B) were found to be differentially expressed, but in an independent validation cohort (20 IBD dysplasia, 20 IBD controls) these results could not be replicated by single RealTime-quantitative PCR (RT-qPCR).

CHAPTER 12

Summary in Dutch (Nederlandse samenvatting)

Inflammatoire darmziekten (IBD), zoals de ziekte van Crohn en colitis ulcerosa, zijn chronische ontstekingsziekten die gekenmerkt worden door opvlammingen, afgewisseld met periodes van remissie. Indien de ziekte langdurig bestaat en een groot gedeelte van de dikke darm betrokken is, bestaat er een verhoogd risico op het ontwikkelen van dikke darmkanker (colorectaal carcinoom, CRC). Op dit moment wordt aangenomen dat in dit geval het cumulatieve risico op dikke darmkanker ongeveer 5% bedraagt na een IBD-ziekteduur van 20 jaar.

Er zijn een aantal factoren die het risico op dikke darmkanker verder vergroten, zoals aanhoudende ziekteactiviteit en de gelijktijdige aanwezigheid van primair scleroserende cholangitis (PSC). Het ontstaan van CRC in een gebied van eerdere ontsteking wordt voorafgegaan door het ontstaan van laaggradige en hooggradige dysplasie. De dysplastische laesies die geassocieerd zijn met colitis zijn in veel gevallen vlak van aard en moeilijk op te sporen.

Indien er bij patiënten minimaal 8 jaar sprake is van IBD en ten minste 30% van het colon is aangedaan, wordt gestart met endoscopische surveillance. Het doel van deze surveillance is het opsporen en verwijderen van gebieden met dysplasie en CRC in een vroeg stadium om daarmee sterfte aan de gevolgen hiervan in deze patiëntgroep te verminderen.

Wij onderzochten door middel van retrospectief en prospectief onderzoek de huidige manier van surveillance bij IBD-patiënten, met als doel de risicostratificatie voor de individuele patiënt te kunnen verbeteren. In het eerste deel van dit proefschrift richten we ons met name op de differentiatie tussen hoog- en laag-risicopatiënten. In het tweede deel ligt de nadruk op het verbeteren van technieken die gebruikt worden om dysplasie op te sporen en te verwijderen.

In hoofdstuk 2 beschrijven we een studie onder 1911 patiënten die surveillance van het colon ondergingen in de periode tussen 2000 en 2015. In het bijzonder richten we ons op de subgroep van patiënten bij wie tevens sprake is van PSC, een chronische leverziekte die geassocieerd is met IBD. Patiënten met zowel PSC als IBD hebben een nog hoger risico om CRC te ontwikkelen. Bij patiënten bij wie reeds laaggradige

dysplasie (LGD) was ontdekt, bleek er ook een hogere incidentie van CRC te bestaan tijdens follow-up. Daarnaast bleek dat bij PSC-patiënten vaker sprake was van LGD die niet goed te visualiseren was. Patiënten met PSC-IBD vormen dus een hoog risicogroep, bij wie intensievere surveillance dient plaats te vinden. Het mechanisme achter dit verhoogde risico is nog niet volledig opgehelderd, maar zou te maken kunnen hebben met de mate van ontstekingsactiviteit of de samenstelling van gal in het colon.

Op dit moment bevelen internationale richtlijnen aan om IBD-surveillance uit te voeren met behulp van chromoendoscopie (CE), waarbij tijdens de coloscopie blauwe kleurstof wordt aangebracht op het oppervlak van de mucosa. Eerdere onderzoeken hebben aangetoond dat met behulp van CE meer dysplastische laesies worden opgespoord dan met standaard-definitie wit licht endoscopie (WLE). In hoofdstuk 3 werden bij 159 patienten laesies met LGD in kaart gebracht en onderverdeeld naar de endoscopische techniek waarmee zij waren gevisualiseerd (CE, standaard-definitie WLE en hoge-definitie WLE). Bij follow-up waren er tussen deze groepen geen significante verschillen in het risico op CRC. Bovendien was bij deze patiënten met LGD het totale aantal CRC's dat optrad laag (omgerekend 1.34 per 100 jaar).

In het vierde hoofdstuk richtten we ons op de categorie patiënten die IBD-surveillance ondergaan, maar desondanks een relatief laag risico hebben op CRC. Hiervoor hadden we een database samengesteld met patiënten uit Nederland, de Verenigde Staten en Canada, die allen routinematig endoscopische surveillance ondergingen. Onder de patiënten die geen hoog-risico kenmerken lieten zien, werd een indeling gemaakt naar de bevindingen bij de eerst bekende coloscopie. Indien bij deze patiënten bij de index coloscopie geen dysplasie, stricturen, pseudopoliepen of tekenen van ontsteking werden gezien, werd deze coloscopie geclassificeerd als 'negatief'. Patiënten bij wie achtereenvolgens twee negatieve coloscopieën hadden plaatsgevonden, trad geen hooggradige dysplasie of CRC op tijdens de follow-up. Bij de overige patiënten varieerde de incidentie tussen de 0.29 en 0.76 per 100 jaar. Patiënten met twee achtereenvolgende negatieve coloscopieën en geen andere risicofactoren komen dus wellicht in aanmerking voor minder intensieve surveillance.

Hoofdstuk 5 beschrijft een studie bij patiënten met IBD die een colectomie hebben ondergaan. Aangezien hierbij het grootste deel van het colon verwijderd wordt, vallen deze patiënten doorgaans niet meer onder de subgroep die reguliere IBD-surveillance aangeboden krijgt. Toch is het risico op het ontwikkelen van CRC niet geheel verdwenen, indien hierbij het rectum niet verwijderd wordt. Wij brachten in deze studie 250 patiënten met IBD in kaart bij wie een zogenaamde rectumstomp ten minste 12 maanden aanwezig is geweest. Gedurende de follow-up periode, bleek het risico op een rectumcarcinoom 3.9 per 1000 jaar. In het tweede deel van de studie onderzochten we de niet-maligne complicaties na colonchirurgie bij IBD-patiënten. Patiënten met een rectumstomp bleken geen meetbare verschillen in kwaliteit van leven te hebben ten opzichte van patiënten bij wie ook het rectum was verwijderd. In deze laatstgenoemde groep was er wel sprake van een hogere frequentie van urologische en seksuele problematiek. Dit maakt de beslissing om het rectum te behouden, dan wel chirurgisch te verwijderen, lastig. Hoewel de afwegingen hierbij persoonlijk zijn, dient bij hoog risicopatiënten (zoals patiënten die eerder dysplasie hebben gehad) de drempel tot het preventief verwijderen van het rectum lager te zijn.

Doordat dysplastische laesies steeds beter te visualiseren zijn, neemt de kans op het succesvol verwijderen van dysplasie toe. Een van de aanbevelingen bij het endoscopisch verwijderen van dysplasie, is het nemen van biopten van de omliggende mucosa. Aangezien deze aanbeveling niet wordt onderbouwd door enig bewijs, verrichtten wij in hoofdstuk 7 een analyse naar de opbrengst van deze biopten. Hiervoor brachten we 140 dysplastische laesies in kaart waarbij omgevingsbiopten waren genomen. Het aantal afgenomen omgevingsbiopten per laesie varieerde tussen 1 en 6 en in slechts 5% van de gevallen was er sprake van dysplasie in deze biopten. Daarbij hadden deze bevindingen geen aantoonbare consequenties en werden er bij follow-up geen hooggradige dysplasie of CRC gevonden. Met het oog op deze resultaten dient naar onze mening het routinematig afnemen van omgevingsbiopten te worden heroverwogen.

Naast verbeteringen in het visualiseren van dysplasie, nemen ook de mogelijkheden met betrekking tot endoscopische resectietechnieken toe. Hoofdstuk 8 beschrijft

een groep patiënten bij wie IBD-geassocieerde dysplasie met een omvang van meer dan 2cm endoscopisch werd verwijderd. Doordat bij deze patiënten het colon is beschadigd door eerdere ontstekingsactiviteit, waren de procedures technisch moeilijk en dienen deze alleen worden uitgevoerd door endoscopisten met ruime ervaring. Daarnaast was er bij follow-up in enkel geval sprake van terugkerende laesies, waarvoor herhaalde endoscopische resectie of chirurgische resectie noodzakelijk was. Het kan daarom geconcludeerd worden dat de endoscopische verwijdering van grote laesies in selecte gevallen een veilige manier is om chirurgische resectie te omzeilen of eventueel uit te stellen.

In hoofdstuk 6 en 9 bespreken we de huidige moderne endoscopische technieken die worden toegepast bij IBD-surveillance. Veruit de meest ingrijpende verandering is de introductie van CE geweest, nadat bleek dat met deze techniek meer dysplasie kan worden opgespoord dan met standaard-definitie WLE. Hoewel de richtlijnen CE op dit moment aanbevelen als de techniek van voorkeur bij IBD-surveillance, bestaat er toch discussie over de meerwaarde van CE bij het gebruik van de inmiddels gangbare moderne apparatuur. Toekomstige studies dienen uit te wijzen of CE inderdaad zijn positie als gouden standaard behoudt. Daarnaast is er behoefte aan minder invasieve manieren van screening, bijvoorbeeld met behulp van biomarkers in bloed of ontlasting.

Een molecuul dat mogelijk als biomarker kan fungeren is microRNA, een vorm van niet-coderend RNA dat onder andere voorkomt in bloed. In onderzoek bij andere patiëntpopulaties konden met behulp van microRNA-profielen prognostische en diagnostische uitspraken worden gedaan. In hoofdstuk 10 werd in een prospectieve setting de waarde van microRNAs als non-invasieve markers van dysplasie onderzocht. Hiervoor werd bij 42 patiënten de expressie van 758 microRNAs in kaart gebracht en vergeleken tussen IBD-patiënten met en zonder dysplasie. Hoewel er voor vijf microRNAs een verschil in expressie kon worden gedetecteerd, werden deze resultaten niet gevalideerd in een tweede onafhankelijke groep patiënten.

CHAPTER 13

General discussion and future perspectives

GENERAL DISCUSSION

IBD is a relatively common, chronic disease, often manifesting early in life. The association between longstanding colitis and CRC has been established for a long time, yet the pursuit of finding an optimal way to prevent this much feared complication is still ongoing.[1] This thesis explores strategies aimed at balancing risk reduction and unnecessary surveillance procedures.

Over the years, our understanding of the incidence, risk factors and endoscopic management of colitis-associated neoplasia and CRC has shifted in a number of ways:

Traditional vs. contemporary risk estimates

Currently, the incidence of CRC in patients with colitis is lower than previously estimated. Earlier studies reported disturbing cumulative CRC rates of up to 30%, but population-based estimates in the current era show a lower risk.[2] Currently, the cumulative risk of CRC is estimated to be 1%, 2%, and 5% after 10, 20, and >20 years disease duration, respectively.[3,4] It is not entirely clear which factors are responsible for this trend, but both improvements in medical therapy resulting in decreased cumulative inflammatory burden and more effective endoscopic surveillance and management might play a role here.

Lesion visibility and endoscopic resection

The way colitis-associated dysplasia is classified endoscopically and histologically has also evolved. Terms like adenoma-like mass (ALM) and dysplasia-associated lesion or mass (DALM) are now outdated.[5,6] The majority of reports on the natural history of LGD in IBD patients originate from an era in which most dysplasia was considered macroscopically invisible and endoscopically unresectable. Consequently, the occurrence of CRC during follow-up was considered to originate from neoplastic progression of these precursor lesions. Since almost all dysplastic lesions are endoscopically visible using modern equipment and invisible dysplasia (previously described as 'flat' dysplasia) is an increasingly rare occurrence, endoscopists now primarily distinguish between resectable and unresectable dysplasia. While the

natural history of dysplasia in IBD is still insufficiently understood, over the years, large studies have shown that completely resected lesions do not result in a significantly increased CRC risk.[7,8] A recent estimate of CRC risk in patients with LGD showed a pooled annual incidence of 0.8%, based on data from 14 studies with rates ranging from 0.2% to 15.4%.[9]

Similarly, random biopsy protocols are being abandoned and a shift towards more targeted surveillance has been made. And finally, with endoscopic therapy becoming more effective, colectomies can increasingly be avoided.[10]

The modulation of cancer risk by the cumulative burden of inflammation over time.

IBD is a chronic illness and over the course of the disease, patients experience periods of relapse and remission in varying degrees. The severity, extent and duration of inflammatory activity differs considerable between patients. Thanks to improved treatment strategies and the introduction of new, highly effective medical therapies, a large number of patients now experience prolonged periods of remission, resulting in a lower risk of CRC. Patients in whom disease is more refractory to medical therapy have persistent (chronic) inflammation, and therefore carry a higher CRC risk.[11–13] Consequently, the amount of mutagenic damage and risk of developing CRC is variable and requires for a more personalized risk assessment.

A number of other risk factors should be taken into account when planning surveillance intervals, such as PSC and family history of CRC. Attempts have been made to develop more personalized risk prediction scores, for example by estimating the individual inflammatory burden for each patient. Recently, some risk factors have lost ground as individual predictors, and are thought to be surrogate measures for inflammatory burden, such as the presence of post-inflammatory polyps.

Current risk stratification tools aim to make an attempt at offering patients a surveillance program that is proportional to their actual risk, but do so in a crude and suboptimal way. For example, guidelines prescribe using the findings from the previous surveillance colonoscopy to plan further follow-up. This practical and easy approach, however, cannot accurately quantify the mutational burden in a colon of a

patient with IBD colitis. In the future, a comprehensive scoring system that is capable of more precise estimations of the inflammatory burden is needed. Incorporating additional data from previous endoscopic procedures could be a promising strategy to improve the performance of a surveillance program. A recent study by Choi et al. has done this by compiling a pragmatic score derived from the severity of endoscopic inflammation on multiple preceding colonoscopies and duration between them.[14] This score was able to give a more accurate risk stratification, without the need of additional procedures.

The results presented in **Chapter 4** reinforce these findings, since patients in sustained remission were found to have a very low risk of advanced neoplasia. Similarly, as seen in **Chapter 2**, active inflammation (coded as a time changing covariate) was significantly associated with advanced neoplasia risk.

These results underscore that the duration of active inflammation is more important than the formerly used total disease duration (i.e. the amount of time since disease onset, irrespective of being in remission or suffering from disease activity). In a similar way, other endoscopic features of previous inflammation, such as the presence of pseudopolyps, may similarly be a diffuse proxy measure of cumulative inflammation.[15]

A novel approach for assessing the cumulative mutational burden in IBD is by tissue sampling of the affected part of the colon. Toiyama et al. investigated whether methylation status in a panel of microRNAs in tissues from UC patients could be used as a tool for identifying patients at high risk for CRC.[16] They were successful in identifying and validating malignancy- and age-related patterns of microRNA methylation in rectal tissue.

Traditionally, a distinction has been made between colitis-associated CRC and sporadic CRC. Recent data further show that this distinction can be made based on the type of recurrent mutations present, as well as the differential mutation frequency. Moreover, in colitis associated CRC, the accrual of mutations seems to be dominated by ageing-associated signatures, most likely caused by the high cell turnover in

inflammatory states. The accrual of mutations, of which TP53 mutations are the most well-known, begins long prior to cancer formation in the colonic epithelium.[17]

If a panel of frequent early mutations can be classified, a tool can be developed that helps identifying the early events leading to cancer formation in the inflamed colon.

The evidence for underlying IBD surveillance

The actual efficacy of IBD surveillance in reducing the risk of colitis associated CRC remains controversial. There are no conclusive data showing that surveillance in the setting of long-standing colitis is effective, although circumstantial evidence suggests a survival benefit. Up to now, five observational studies have been performed[18–22], but no RCT is available that compares surveillance to another strategy.[23] It is highly unlikely that such an RCT with a non-surveillance comparison group will be performed. This does not mean that research into IBD surveillance is scarce. To the contrary, a large numbers of high quality RCTs have been performed, comparing different endoscopic techniques in their ability to detect neoplasia ('flagging studies') and subsequently characterize the lesion (endoscopic differentiation). Since the absolute number of CRCs is small, most studies use dysplasia as the primary endpoint. Dysplasia is generally considered the most appropriate surrogate endpoint for CRC risk. Still, simply finding more dysplasia may not be the best quality measure for studying the actual clinical impact of surveillance and potential negative effects of relying on dysplasia as a primary measure of CRC risk is an increase in (possibly unwarranted) colectomies and anxiety among patients and physicians.

In this context, two types of bias should be noted: A test may detect dysplasia earlier than a less sensitive test. Still, this may not affect the survival time of the patient. If the test only accomplishes a longer follow-up time, without actually lengthening patient survival time, this is called *lead-time bias*. In addition, a more sensitive test may predominantly detect more slowly growing lesions, whereas a less sensitive test likely will detect more rapidly progressing lesions, which is called *length-time bias*.

Future trials comparing different endoscopic techniques for dysplasia detection should be adequately powered, randomized (with randomized sequences of

procedures if a tandem study is performed) and make use of modern high-definition equipment.

Ideally, the techniques studied should be shown to improve actual outcomes, beyond just providing early dysplasia detection.

Identifying high risk groups – Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is associated with both UC and CD and is a much-feared complication. Clinically, the colonic inflammation accompanying PSC may be extensive yet mild, leading to a difficult definition of IBD disease onset since symptoms may be absent for a long time.[24]

The increased risk of CRC in IBD patients with concurrent PSC has been thoroughly studied, yet the underlying pathophysiologic mechanisms remain unclear.[25–28] A 2013 study by Boonstra et al. identified PSC patients in 44 hospitals in the Netherlands and found a 10-fold increased CRC risk for PSC-UC patients, compared to UC-only patients.[29] The investigators also found a younger age (more than 20 years earlier) at CRC diagnosis compared to regular IBD patients and the general population.

In the study reported in **Chapter 2**, we found a more than twofold increase in risk for IBD patients with PSC. The increased neoplasia risk for PSC patients persisted after correction for active endoscopic inflammation over time. We used a time-changing covariate (rather than a mean overall score) for inflammatory activity, which allows for multiple data points over time to be incorporated in the model. Interestingly, endoscopic activity measured this way was strongly associated with the risk of neoplasia, in addition to the effect of a concurrent PSC diagnosis.

Most likely, the underlying pathophysiology is multifactorial with roles for gene–environment interactions, the microbiome, as well as epigenetic modifications.

One hypothesis that might explain the increased risk of colitis-associated CRC is an altered composition of bile acid.[30] Notably, a right sided predominance of neoplasia, reported in several studies reinforces this association.[27,31] Secondly, alterations in the colonic microbiome may have procarcinogenic effects on the colonic epithelium,

although there is no direct evidence for this link.[32] There may also be genetic predispositions that induce oncogenic mechanisms in a more direct way. Large genome-wide association studies have shown distinct genetic differences in PSC patients.[33]

The mechanisms proposed above may also lead to the development of morphologically distinct precursor lesions. Our study reinforces earlier findings that in PSC patients, LGD is more often identified in random biopsies, suggesting that these lesions are more inconspicuous or flat-out invisible. Given these findings, we propose that the current trend of omitting random biopsies from the surveillance protocol should not include PSC patients.[34,35]

Our findings suggest that although continued meticulous CRC surveillance with annual colonoscopy is indicated in the absence of dysplasia for patients with PSC-IBD, the detection of LGD or higher-grade pathology should lead to a careful weighing of the pros and cons of a more aggressive therapeutic management, including colectomy.

Identifying high risk groups – patients with low-grade dysplasia

In IBD surveillance, detecting and removing precancerous lesions should ideally result in a reduction of the incidence of future CRC.

The introduction of newer endoscopic techniques and equipment has resulted in increased sensitivity for dysplasia detection.[36,37] Perhaps the most discussed transition in this context has been the introduction of chromoendoscopy (CE), which has shown higher dysplasia detection rates than standard definition WLE.[38] Ideally, the incremental yield in dysplastic lesions would result in a proportional incidence reduction of colorectal cancers. However, since the natural course of colitis-associated dysplasia has not been fully elucidated, it is not possible to automatically make this assumption. Whereas some lesions may grow very rapidly, others may show a more indolent course or even regress over time.

The study described in **Chapter 3** was set up in part to provide data on the clinical significance of the precursor lesions found using different surveillance techniques.

The biologic behavior of the additional lesions found using CE has not been established.[39,40] Some authors have hypothesized that these smaller lesions may be less advanced and therefore may have less malignant potential, yet no evidence in support of this hypothesis has been published to date.

We did not find a significant difference in the risk of advanced neoplasia during follow-up for index lesions detected with either WLE or CE, nor did we find additional risk factors at the time of index colonoscopy that were associated with the occurrence of advanced neoplasia during follow-up. On the basis of these results, we conclude that the risk of advanced neoplasia after the detection of an LGD lesion is not dependent on the endoscopic method used to detect it. However, the low number of advanced neoplastic lesions may have caused a lack of power to detect more subtle differences.

In addition to the nature of the lesion itself, factors that determine the risk of recurrence or advanced neoplasia include the resection technique, operator skill, lesion size, and whether resection has been macroscopically complete. The endoscopic technique used to detect the lesion, could affect some of these factors in varying degrees. For example, the use of CE might lead to a higher estimation of lesion size and better visualization and delineation enabling more complete resections, but could also lead to more indolent lesions being found and resected.

We found a significantly lower incidence rate of advanced neoplasia following the identification of LGD lesions as compared to older cohort studies. However, more recent reports seem to be in line with our estimated overall incidence rate of 1.34 advanced neoplasia cases per 100 patient-years

Other studies have revealed additional characteristics of LGD that are associated with a later diagnosis of HGD or CRC. Choi et al. found non-polypoid lesion appearance (Paris type 0-II , type 0-III or plaque-like), invisible dysplasia, lesion size ≥1 cm, and previous history of indefinite for dysplasia to be significant predictors. A recent meta-analysis by Fumery et al. collected data on 14 surveillance cohorts and found concomitant primary sclerosing cholangitis, invisible dysplasia, distal location, and multifocal LGD to increase the risk of 'progression' to advanced neoplasia.[41]

Invisible dysplasia (sometimes also named flat dysplasia) managed by endoscopic follow-up was an important subgroup in our study, as the incidence rate of advanced neoplasia was highest in these patients. This higher incidence rate may be explained by the fact that residual dysplastic mucosa was undoubtedly present in these patients. Nonetheless, it cannot be excluded that this was the result of a field cancerization effect.

Advanced neoplasia was found to develop infrequently after detection of LGD in patients undergoing endoscopic surveillance for IBD. In our study, LGD detected with either chromoendoscopy or WLE carried a similar risk of advanced neoplasia over time.

Identifying low risk groups

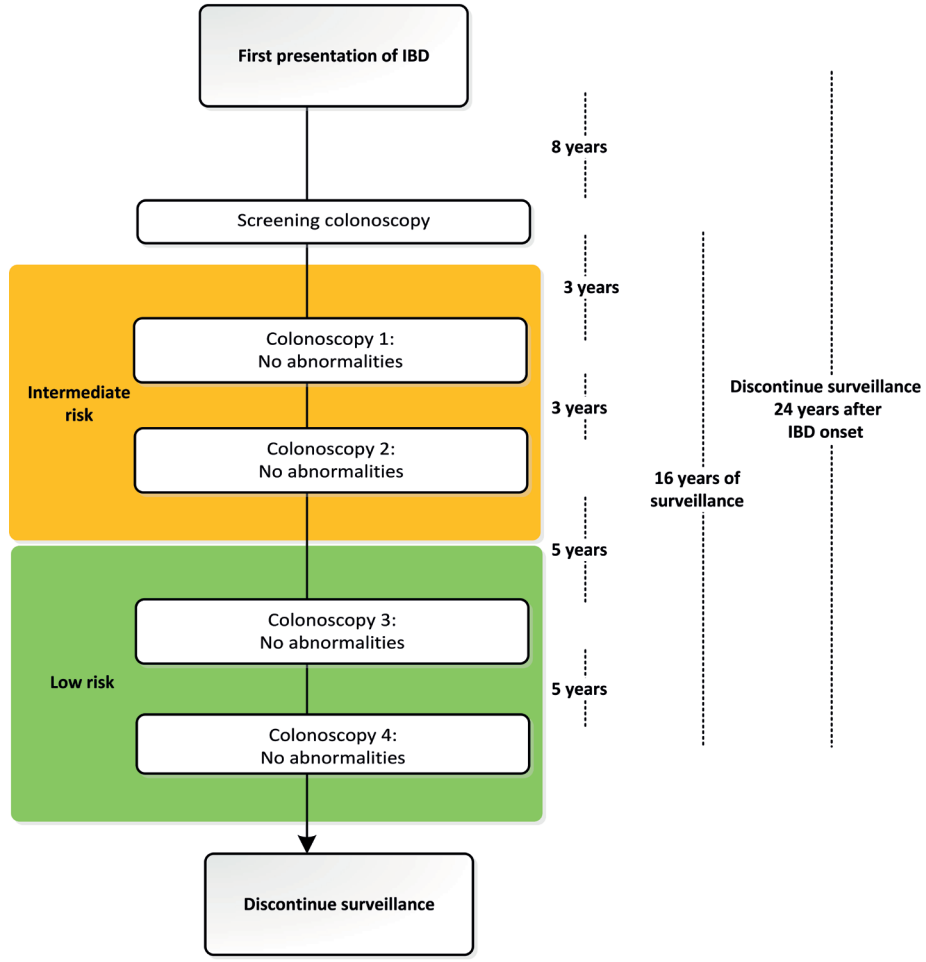
Patients enrolled in IBD surveillance are offered frequent colonoscopies, which results in a cancer screening program that is highly intensive. In contrast, the decreasing incidence of IBD-associated CRC over time results in a lower dysplasia yield per surveillance colonoscopy, thereby affecting the risk-benefit balance of this practice.[42]

In the population undergoing IBD surveillance, recommended intervals for endoscopy range from 1 to 5 years, depending on clinical and endoscopic factors. Unfortunately, the appropriateness of the recommended intervals between colonoscopies in this population has been incompletely studied. In addition, the recommendations are inconsistent geographically as European guidelines advocate a 3-tier system whereas North American guidelines employ a 2-tier system.[43,44] Patients with well-controlled IBD and no additional risk factors fall into the lowest risk category.[45] This category of patients was the focus of **Chapter 4**.

In an effort to maximize statistical power, we established a tri-national and multicenter database, one of the largest to date among this population. The criteria we used to compose the group of interest (the low risk group) were strict. Based on these criteria, less than 30% had high-risk demographics that necessitated yearly colonoscopies. Among the remaining group, over 30% had at least one negative surveillance, which indicates that there is a large group in whom there is a potential for downgrading surveillance intensity leading to cost- and resource-savings.

In the low-risk group with two consecutive negative surveillance colonoscopies, no one developed high-grade dysplasia or CRC during follow-up. These findings suggest that longer surveillance intervals may be appropriate for this patient group.

The updated Dutch guideline on IBD surveillance includes a new suggestion, in which repeated negative examinations may lead the gastroenterologist and patient to consider suspending endoscopic surveillance if no additional risk factors are present.[46] An example of this scenario is shown below (in this case, a patient with intermediate risk at the start of surveillance is chosen as an example). It is important to emphasize that for this scenario, no active inflammation should be present;



Our study did not include a matched cohort of the general population, which would have provided a contextual comparison of the added CRC risk in the low-risk IBD group to the overall background risk of CRC.

Presently, it is unknown how long this low risk is sustained over time. Unfortunately, we had insufficient data to look beyond 5 years, in part because the initial categorizing based on two colonoscopies had already taken up a significant number of follow-up years.

Another important caveat is the fact that we did observe LGD in the follow-up of low-risk patients. This finding alone suggests that ongoing surveillance in these patients

is still needed. As mentioned before, there is a clear need for up-to-date data on incidence, test sensitivity, and natural history of dysplastic lesions.

Our findings suggest that in the absence of additional risk factors for CRC and with at least two consecutive negative exams a longer surveillance interval may be appropriate. This might optimize the resource-to-benefit ratio of surveillance and improve the quality of life for a large percentage of IBD patients enrolled in surveillance.

The need for continued surveillance after colonic resection

Up to Chapter 5, this thesis had focused on the typical subgroup of IBD patients that had not undergone a colectomy and could easily be identified as a candidate for surveillance. However, a significant group of IBD patients need to undergo surgery at some point in their disease course. Nearly one third and more than 70% of the patients diagnosed with ulcerative colitis (UC) and Crohn's disease (CD), respectively, will require surgery.[47,48] A subtotal colectomy with an ileostomy is the procedure of choice when patients are suffering from severe refractory colitis. Typically in UC, the preferred restoration technique is a proctectomy in combination with an ileal pouch-anal anastomosis (IPAA).[49] When pouch surgery is not appropriate or contraindicated, an ileostomy can be a definitive outcome. Due to the potential risk of pelvic nerve damage and pelvic septic complications, the rectum may be left in situ. Likewise, in 25% of patients with colonic CD, an end ileostomy is constructed with a closed rectal stump.

In case a subtotal colectomy has been performed, the risk of developing cancer has not been completely eliminated since a retained rectum continues to carry a risk of malignant transformation. In order to prevent development of rectal stump cancer (RSC), enrolling these patients in a surveillance program should be considered. At this point, clear guideline recommendations are lacking, and surveillance itself is inconsistently performed in daily clinical practice.

In **Chapter 5**, we found a relatively low incidence rate of RSC in our IBD cohort, which is in agreement with previous reports.[50,51] A history of colonic neoplasia was associated with advanced rectal stump neoplasia during follow-up. This suggests

an underlying susceptibility to developing dysplasia which can be explained in several ways. There may be a genetic predisposition to neoplasia formation, but previous colitis (and/or possible field cancerization) might also contribute. We found a high prevalence of diversion colitis, which was in line with previous reports.[52,53] Unfortunately, we had insufficient data to ascertain whether ongoing inflammation of the (out-of-circuit) rectal mucosa further increased the risk of rectal stump cancer.

Decision making for the long-term management of a rectal stump can be difficult, and clinicians should weigh the impact of proctectomy against the risks and disadvantages of a retained rectum.

Aspects in favor of proctectomy include eliminating the risk of rectal stump cancer (and the need for endoscopic surveillance) and relief of symptomatic proctitis. On the other hand, reasons to hold off proctectomy are potential morbidity, particularly urinary and sexual dysfunction, associated with pelvic surgery. In our attempt to compare these two scenarios (proctectomy vs. rectum-in-situ) we did not detect significant differences in quality of life scores. In line with our expectations, slightly more sexual/urinary complaints were reported in the proctectomy group, whereas blood loss/stump related problems were not uncommon in the comparison group.

The decision to perform a completion proctectomy should be made on a case by case basis. Our study does not give universal recommendations, but we do believe a low threshold for proctectomy is justified in patients with a history of colorectal neoplasia or symptomatic diversion colitis.

Guideline committees should also consider offering recommendations for endoscopic or radiographic surveillance and call for more prospective data to be gathered in this often-overlooked group of patients.

RSC has a low incidence rate, with patients with a history of colonic neoplasia carrying the highest risk of developing this severe complication.

Improving screening sensitivity through advanced endoscopic imaging

Flagging studies

Endoscope technology has advanced significantly over time, with high-definition equipment now being standard of care. At this moment, dye chromoendoscopy (CE) with targeted biopsies is still considered to be the 'gold standard' endoscopic technique for IBD surveillance, as described in European guidelines and the SCENIC consensus statement. However, the studies on which these recommendations are based do show heterogeneity and for a large part, standard definition WLE was used in the comparator arms.

High-definition endoscopes and equipment produce more superior image signals (measured as number of pixels) and offer a broader field of vision (170 degrees), enhancing the potential for lesion detection and characterization.[54,55]

RCTs have shown a small benefit of CE over standard-definition WLE (SDWLE).[56–58] However, the available evidence comparing it to high-definition WLE (HDWLE) is weak and no RCT has shown superiority of CE over HDWLE.[59]

A number of biases may distort the evidence comparing different endoscopic techniques. First, in most studies, performing CE leads to additional procedure time. This increased time could lead to more time inspecting the mucosa, providing a direct benefit for dysplasia detection.[60,61] Second, some studies were designed as a tandem-trial, in which the two techniques are performed back-to-back in the same patient. However, since chromoendoscopy is restricted to being the second procedure in line (once dye spraying has occurred, white light endoscopy is no longer possible), bias is inevitably introduced.

Possibly, pancolonic dye-spraying may become redundant in the future if adequately trained endoscopists are able to perform optimal surveillance using HD equipment. Considering the barriers to the wide implementation of CE, such as the learning curve, cost and increased time, this would be welcomed by many gastroenterologists.

When lesions have been targeted, irrespective of the technique, dye spraying is routinely used as an adjunct to more accurately define lesion borders and assessing surface patterns.

New generations of equipment frequently contain integrated optical technologies designed to enhance characteristics of the image produced, for example NBI (Olympus) and iSCAN (Pentax). These technologies aim at enhancing certain details of the tissue surface or presence of blood vessels without application of dye. Recently, iSCAN has been redeveloped to include Optical Enhancement (OE) filters, but this technique has not yet been studied in the detection of colitis-associated dysplasia.

The first studies with NBI, one of which was performed in a Dutch setting, showed unfavorable results for the technique.[62–64] A more recent study showed somewhat better results, finding equal detection rates to HD-WLE.[65] A recent meta-analysis, published in 2019, concluded NBI was not superior to WLE.[66] Given the lack of superiority, NBI has not been implemented as a flagging technique for IBD surveillance.

Autofluorescence imaging was discussed in **Chapter 6**. Recently, the results of the FIND-UC trial have been published by Vleugels et al.[67] In this prospective RCT, AFI was compared to chromoendoscopy in 210 patients with longstanding UC. The number of patients in whom dysplasia was detected was significantly lower in the AFI arm (12%) compared to the chromoendoscopy arm (19%). Therefore, the authors state that AFI should not be further investigated as an alternative dysplasia detection method.

Endoscopic classification

Endoscopic characterization of lesions suspected of being neoplastic is as important as it is difficult. The terms DALM (dysplasia associated lesion or mass) or ALM (adenoma-like mass) have been discarded and have been replaced by a the more helpful distinction between endoscopically resectable and non-resectable lesions. Guidelines also advocate the use of endoscopic descriptors modified from the Paris

classification.[68] Other characteristics for predicting lesion histology have so far not been advocated.

In general, Kudo pit patterns III-V seem to be associated with neoplasia[69,70], yet there is moderate interobserver agreement in the assessment of Kudo pit patterns.[71]

Recently, a group of authors has developed a new classification system (Frankfurt Advanced Chromoendoscopic Ibd LEsions, FACILE), in which flat shape, irregular surface, vascular pattern and signs of inflammation predicted dysplasia. Nonetheless, its added value in daily practice remains to be seen.[69,72] Lastly, endoscopic tri-modal imaging, in which WLE, NBI and AFI are combined, was investigated in 2018 as a tool for endoscopic characterization (differentiating dysplasia from benign lesions). The authors found limited added value for real-time prediction of dysplasia, although there was a high negative predictive value making it potentially useful in excluding dysplasia.[73]

Improved visualization permits a reduction in unnecessary tissue sampling

Colitis associated dysplasia is considered difficult to identify and hard to classify and resect. Endoscopic surveillance has long been hindered by a lack of sensitive screening methods, resulting in concerns about missed lesions. The risk of multifocality and missed dysplasia led gastroenterologists to resort to a random biopsy protocol, taking numerous (>30) nontargeted biopsies during each surveillance colonoscopy. However, this strategy can be considered a safety measure to overcome the shortcomings of older endoscopic techniques and has been challenged in recent times.[35]

An RCT performed by Watanabe et al. compared targeted-only protocol, compared to a targeted approach supplemented with a random biopsy protocol.[74] The targeted-only approach was non-inferior to the random biopsy group. In fact, the targeted approach resulted in an even higher dysplasia detection rate. Possible explanations include a distraction from meticulous inspection when performing a time-consuming random biopsy protocol. Furthermore, bleeding from random biopsies may compromise mucosal inspection. Another recent study by Moussata et

al. confirmed these findings in a prospective study among 1000 patients.[75] However, they did point to risk factors for neoplasia that was detected by random biopsies, i.e. a personal history of neoplasia, but also concomitant PSC or a tubular colon. In patients in whom these risk factors were lacking, the likelihood of detecting neoplasia in random biopsies was close to zero and should be omitted.

The concern about missed lesions and incomplete resections has also resulted in guideline recommendations prescribing biopsies to be taken from the area adjacent to the resected lesion. These semi-random biopsies (taken randomly from a targeted area) are mainly aimed at detecting invisible dysplasia, but the recommendations are not based on any evidence.

While the paradigm of endoscopic surveillance is moving away from random biopsies to a targeted approach, our hypothesis in **Chapter 7** was that biopsies of the surrounding mucosa would no longer be needed as a routine measure. We found a very low diagnostic yield for biopsies of the surrounding mucosa. Moreover, in patients with adjacent biopsies showing dysplasia there was no measurable impact on clinical outcomes.

The most straightforward explanation for positive biopsies of surrounding mucosa is a limited visibility of the lesion borders (before resection) leading to residual dysplastic tissue (after resection), Another explanation is the use of the same biopsy equipment for lesions as for surrounding mucosa. Theoretically, using the same biopsy forceps for random, semi-random, and targeted biopsies in the same session may cause residual tissue of one lesion to be incorrectly allocated to another lesion. However, this has not been shown and is estimated to be very rare.

Cases of surrounding mucosa dysplasia have been demonstrated in SD WLE, HD WLE, and chromoendoscopy procedures. We found a lack of clinical consequences related to taking biopsies of surrounding mucosa. Our findings were later confirmed by two other studies[76,77], which underscores that surrounding biopsies are neither useful nor cost-effective as a routine measure. Histologic assessment of the surrounding

mucosa may still be able to provide additional information when there is uncertainty about the distinction between sporadic and colitis-associated neoplasia.

Another theoretical framework that might justify non-targeted biopsies is that of so-called field cancerization. As shown in a recent study by Baker et al, adjacent non-cancerous mucosa from CRC does show an accrual of mutations[17], which indicates a field effect. This concept suggests that (epi)genetic changes may occur throughout the colonic epithelium in patients with colitis, increasing the baseline risk of developing neoplasia. These changes, which pre-date the development of dysplasia, may serve as an indication for the future risk of neoplasia in individual patients. If assays can be developed to identify these changes and adapt them into personalized risk scores, then the added value of random biopsies should be reappraised.

With better means of endoscopic visualization, IBD surveillance is moving away from a non-targeted towards a more targeted approach, supported by evidence showing that routinely taking random biopsies and biopsies from surrounding mucosa is no longer useful.

Avoiding colectomy through effective and safe endoscopic management

As discussed earlier, we now classify lesions as either resectable or non-resectable, as opposed to older terminologies (i.e. ALM or DALM).[7,78] Technological advances are driving the possibilities of endoscopic therapeutic management into new territories. Endoscopic mucosal resection (EMR) is increasingly adopted to remove colitis-associated dysplasia and the recent introduction of endoscopic submucosal dissection (ESD) further expands the possibilities for local resection in IBD patients with precancerous lesions. These techniques may offer an alternative to colectomy ('colon sparing management').

Nevertheless, management of colonic neoplasia in patients with IBD is known to be challenging due to inflammatory damage, most notably fibrosis. Also, the subsequent risk of metachronous neoplasia should be considered when opting for a non-surgical approach.

Irrespective of size or morphologic features, en-bloc resection of dysplastic lesions should be the intent. Neoplastic lesions are often detected during a surveillance procedure and endoscopists might be inclined to immediately remove this lesion with EMR. Unfortunately, in case of a large or non-pedunculated lesion, EMR might result in piecemeal resection, increasing the risk of residual tissue. Piecemeal resection also hampers the histopathological assessment and thereby the decision-making process. For en-bloc resection, especially in the setting of IBD, ESD is probably the more appropriate technique. As of yet, ESD is considered experimental in the setting of colitis-associated dysplasia. Recently, studies are emerging showing the safety and long-term outcomes of ESD in this setting. While the procedure is considered difficult, the limited results published to date are promising.

The results of our small explorative study are in line with other similarly sized reports. Iacopini et al. studied the safety of ESD in nine lesions >20mm in size and found a curative resection rate of 70% after 2 years.[79] Suzuki et al., found just one recurrence after 32 procedures with a median follow-up of 33 months.[80] Lastly, Kinoshita et al. performed a similar study in 25 patients and identified 5 patients (25%) with a non-curative resection who underwent additional surgery.[81]

It should be noted that submucosal fibrosis, secondary to long-standing mucosal inflammation, and problems with lesion demarcation both render these procedures more challenging. Therefore, we feel that ESD should be performed by endoscopists with specialized training and adequate experience.

The detection and management of colonic neoplasia in patients with IBD is known to be challenging. The recent introduction of advanced endoscopic techniques broadens the possibilities for IBD patients with precancerous lesions and may offer an alternative to colectomy.

Moving towards less invasive screening methods

Endoscopic surveillance is burdensome and suboptimal, but up to now, less-invasive alternative screening methods are not available. In our aim to identify non-invasive biomarkers to identify patients at high risk for neoplasia development, we

studied differences in expression of microRNAs (miRNAs) in plasma of IBD patients undergoing surveillance.

Along a wide range of diseases, the role of miRNAs has been explored as diagnostic and prognostic biomarkers. In sporadic CRC and its precursor lesions, a differential expression of circulating microRNAs has been reported.[82–84] The pathways in which miRNAs play a role could be tumor-suppressive, anti-inflammatory or, for a large number of miRNAs, largely unknown. Although there is little consistency in the main findings across these studies, most authors are optimistic about the possibilities in using miRNAs as actual tools in patient care. For colitis-associated cancer, no full reports on blood-based miRNAs had yet been published.

Although we identified five microRNAs to be differentially expressed in patients with colitis-associated dysplasia, we were unable to validate the increased expression of these microRNAs in a confirmation cohort. Several factors might explain these negative findings. First, a large number of factors, both related and unrelated to IBD, could affect miRNA levels and distort the results. Even though patients were matched on age and sex, and originated from the same standardized cohort, we were unable to completely eliminate variability with respect to inflammation, weight and medication use.

Since we used blood based markers instead of tissue based markers, another possibility for the negative findings is that the stage of progression was not sufficient enough to produce measurable differences in miRNA levels. However, if miRNA profiling is only effective in advanced stage cancers, its role in preventative strategies is (obviously) limited.

We also considered the possibility of study-related technical factors to be responsible for a failure to detect a possible difference. However, we followed the manufacturer's protocols carefully and used a workflow that had been shown to generate positive results in other fields. miRNAs received a great deal of attention over the past decade, but given our findings, we conclude that miRNAs do not provide added value in the current setting of IBD surveillance.

Though circulating microRNAs do not seem to meet expectations for their application in IBD surveillance, we look forward to research into other technologies with a potential to improve and streamline surveillance. Several groups are currently investigating biomarkers in blood, feces and tissue.[16,85–89]

There is need for a robust non-invasive biomarker capable of identifying IBD patients at risk for neoplasia, but this need is currently unmet.

Statements on future research efforts

In **Chapter 9** of this thesis, practical advice is provided on the planning and performance of surveillance colonoscopies. Although the current guidelines provide a framework for day-to-day practice, gastroenterologists often encounter scenarios for which no formal recommendations exist.

We specifically stressed the importance of preparation (optimal bowel preparation and remission induction), lesion assessment (discussion in a multidisciplinary team) and endoscopic resection (training and operator experience).

Finally, we provided several suggestions for the research agenda for the next 5-10 years:

<u>High quality, prospective evidence on risk categories, working towards personalized risk assessments</u>

The 3-tier system used for planning surveillance intervals in Europe is based on assignment of patients to high-risk (1-year interval), intermediate risk (3-year interval) and low-risk (5-year interval) groups. The evidence for this risk hierarchy is however limited. Ideally, each patient should receive a personalized risk score, based on solid evidence from large prospective cohorts, comparable to prognostic tools used in, for example, breast cancer or cardiovascular disease.[90]

To this end, we not only need large study populations, but also detailed measurements of disease characteristics and endoscopic interventions over time. This requires substantial resources.

<u>International collaboration, dataset harmonization and prospective registration</u>

Since the incidence of colitis-associated CRC is relatively low, large longitudinal studies are needed with a long follow-up time, in order to attain adequate statistical power. Some of the studies in this thesis have been specifically designed to increase power by collaboration between centers. To further improve this field of research, we need large prospective registries that provide data that can be harmonized and/or aggregated into pooled meta-analyses. Large-scale, multinational projects could accelerate the much-needed improvements in risk prediction and management.

Develop ways of quantifying cumulative (mutagenic) inflammatory damage.

In this thesis, we have discussed the role of cumulative inflammatory activity and ways of scoring this risk clinically. Based on repeated endoscopic examinations, the inflammatory activity over time can be quantified, albeit crudely, and this has been shown to reflect neoplasia risk.[91] There is a need for new scoring systems that cover more information than current scoring systems (which are based solely on the most recent endoscopy). Ideally, the scoring of accumulated inflammatory damage should be condensed to a single measurement, for example an index biopsy that allows for analyzing methylation markers that reliably predict the risk of malignant progression.

Differentiation between dysplasia types using biomolecular and genetic markers. Harmonize variation in terminology for dysplastic lesions.

Older, confusing terminologies have been replaced by more straightforward definitions or descriptions. Dysplastic lesions are best categorized as resectable or unresectable and best described using the terminology of the Paris classification, although some lesions may not be categorized easily. In histopathologic classification, the clinical impact of molecular pathology and immunologic staining remains unclear. Whether next-generation and whole-gene sequencing could add more clarity in lesion assessment, remains to be seen.

A category of precursor lesions that has increasingly become recognized is the serrated pathway.[92] Since these lesions are not uncommon in the IBD population, more research is needed on their malignant potential, specifically in relation to chronic inflammation.

Determine whether HDWLE is truly comparable to CE surveillance.

The superiority of CE over SD-WLE has been widely shown, but whether CE has additional benefit over HD-WLE is controversial and currently still under investigation. Perhaps, for average to low risk patients, careful surveillance with HD-WLE may be all that is necessary. Studies investigating differences between index and repeated surveillance colonoscopies is also lacking.

Develop strategies for reducing unnecessary examinations.

IBD surveillance currently largely depends on patients' willingness to undergo repeated examinations. In Chapter 4, we showed that certain patient groups carry a very low risk of malignancy and surveillance intervals may probably safely be extended. Reducing the burden of intensive screening in IBD surveillance is of major importance; a properly designed, effective surveillance program should incorporate appropriate definitions of the population at risk. Second, efforts should be made to shift screening from invasive to less-invasive investigations.

Develop and validate non-invasive methods for risk assessment and neoplasia detection.

In this thesis, we explored the value of circulating microRNAs as a biomarker for dysplasia. This was a negative study, but other candidate biomarkers might have the potential to improve or simplify IBD surveillance in drastic ways.

Most biomarker research to date has focused on colonic biopsies obtained during colonoscopies. Potentially useful assays on tissue include mutational signatures[93], methylation status[16,94,95], and proteomics profiling.[96,97] Ideally a single rectal biopsy, which can be retrieved with minimal preparation and discomfort, would provide sufficient information on neoplasia risk of the entire colon.

A potentially attractive technique is stool DNA testing, which reportedly has shown promising results as a non-invasive screening measure for sporadic CRC and is now being studied in colitis-associated CRC.[86,98]

Obviously, blood represents the ideal biomaterial due to its easy accessibility. Potential targets include DNA-based markers[99,100], protein markers[101,102], and metabolomics profiling[103], yet no large-scale studies on colitis associated CRC have yet been published.

If early results can be validated in large, independent studies, these biomarkers have the potential to extend endoscopic surveillance intervals. Possibly, these might even replace colonoscopy as the first-line investigation.

REFERENCES

1. Beaugerie, L. & Itzkowitz, S. H. Cancers complicating inflammatory bowel disease. *N. Engl. J. Med.* **372,** 1441–52 (2015).
2. Jess, T. *et al.* Decreasing risk of colorectal cancer in patients with inflammatory bowel disease over 30 years. *Gastroenterology* **143,** 375-81–4 (2012).
3. Lutgens, M. W. M. D. *et al.* Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm. Bowel Dis.* **19,** 789–99
4. Söderlund, S. *et al.* Decreasing time-trends of colorectal cancer in a large cohort of patients with inflammatory bowel disease. *Gastroenterology* **136,** 1561-7-9 (2009).
5. Chiu, K., Riddell, R. H. & Schaeffer, D. F. DALM, rest in peace: a pathologist's perspective on dysplasia in inflammatory bowel disease in the post-DALM era. *Mod. Pathol.* **31,** 1180–1190 (2018).
6. Laine, L. *et al.* SCENIC International Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease. *Gastroenterology* **148,** 639–651.e28 (2015).
7. Vieth, M., Behrens, H. & Stolte, M. Sporadic adenoma in ulcerative colitis: endoscopic resection is an adequate treatment. *Gut* **55,** 1151–1155 (2006).
8. Wanders, L. K. *et al.* Cancer risk after resection of polypoid dysplasia in patients with longstanding ulcerative colitis: a meta-analysis. *Clin. Gastroenterol. Hepatol.* **12,** 756–64 (2014).
9. Fumery, M. *et al.* Incidence, Risk Factors, and Outcomes of Colorectal Cancer in Patients With Ulcerative Colitis With Low-Grade Dysplasia: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* **15,** 665–674.e5 (2017).
10. Odze, R. D., Farraye, F. A., Hecht, J. L. & Hornick, J. L. Long-term follow-up after polypectomy treatment for adenoma-like dysplastic lesions in ulcerative colitis. *Clin. Gastroenterol. Hepatol.* **2,** 534–41 (2004).
11. Gupta, R. B. *et al.* Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* **133,** 1099-105–1 (2007).
12. Rubin, D. T. *et al.* Inflammation is an independent risk factor for colonic neoplasia in patients with ulcerative colitis: a case-control study. *Clin. Gastroenterol. Hepatol.* **11,** 1601-8–4 (2013).
13. Rutter, M. *et al.* Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* **126,** 451–9 (2004).
14. Choi, C.-H. R. *et al.* Cumulative burden of inflammation predicts colorectal neoplasia risk in ulcerative colitis: a large single-centre study. *Gut* **68,** 414–422 (2019).
15. Mahmoud, R. *et al.* No Association Between Pseudopolyps and Colorectal Neoplasia in Patients With Inflammatory Bowel Diseases. *Gastroenterology* **156,** 1333–1344.e3 (2019).

16. Toiyama, Y. *et al.* A Panel of Methylated MicroRNA Biomarkers for Identifying High-Risk Patients with Ulcerative Colitis-associated Colorectal Cancer. *Gastroenterology* (2017).

17. Baker, A.-M. *et al.* Evolutionary history of human colitis-associated colorectal cancer. *Gut* gutjnl-2018-316191 (2018). doi:10.1136/gutjnl-2018-316191

18. Choi, P. M., Nugent, F. W., Schoetz, D. J., Silverman, M. L. & Haggitt, R. C. Colonoscopic surveillance reduces mortality from colorectal cancer in ulcerative colitis. *Gastroenterology* **105,** 418–24 (1993).

19. Ananthakrishnan, A. N. *et al.* Colonoscopy Is Associated With a Reduced Risk for Colon Cancer and Mortality in Patients With Inflammatory Bowel Diseases. *Clin. Gastroenterol. Hepatol.* **13,** 322–329.e1 (2015).

20. Karlen, P. *et al.* Is colonoscopic surveillance reducing colorectal cancer mortality in ulcerative colitis? A population based case control study. *Gut* **42,** 711–714 (1998).

21. Lashner, B. A., Kane, S. V & Hanauer, S. B. Colon cancer surveillance in chronic ulcerative colitis: historical cohort study. *Am. J. Gastroenterol.* **85,** 1083–7 (1990).

22. Lutgens, M. W. M. D. *et al.* Colonoscopic surveillance improves survival after colorectal cancer diagnosis in inflammatory bowel disease. *Br. J. Cancer* **101,** 1671–5 (2009).

23. Bye, W. A. *et al.* Strategies for Detecting Colorectal Cancer in Patients with Inflammatory Bowel Disease: A Cochrane Systematic Review and Meta-Analysis. *Am. J. Gastroenterol.* **113,** 1801–1809 (2018).

24. Weismüller, T. J. *et al.* Patient Age, Sex, and Inflammatory Bowel Disease Phenotype Associate With Course of Primary Sclerosing Cholangitis. *Gastroenterology* **152,** 1975–1984.e8 (2017).

25. Broomé, U., Löfberg, R., Lundqvist, K. & Veress, B. Subclinical time span of inflammatory bowel disease in patients with primary sclerosing cholangitis. *Dis. Colon Rectum* **38,** 1301–5 (1995).

26. Kornfeld, D., Ekbom, A. & Ihre, T. Is there an excess risk for colorectal cancer in patients with ulcerative colitis and concomitant primary sclerosing cholangitis? A population based study. *Gut* **41,** 522–5 (1997).

27. Shetty, K., Rybicki, L., Brzezinski, A., Carey, W. D. & Lashner, B. A. The risk for cancer or dysplasia in ulcerative colitis patients with primary sclerosing cholangitis. *Am. J. Gastroenterol.* **94,** 1643–9 (1999).

28. Navaneethan, U. *et al.* Duration and severity of primary sclerosing cholangitis is not associated with risk of neoplastic changes in the colon in patients with ulcerative colitis. *Gastrointest. Endosc.* **75,** 1045–1054.e1 (2012).

29. Boonstra, K. *et al.* Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. *Hepatology* **58,** 2045–2055 (2013).

30. Barrasa, J. I., Olmo, N., Lizarbe, M. A. & Turnay, J. Bile acids in the colon, from healthy to cytotoxic molecules. *Toxicol. In Vitro* **27,** 964–77 (2013).

31. Claessen, M. M. H. *et al.* More right-sided IBD-associated colorectal cancer in patients with primary sclerosing cholangitis. *Inflamm. Bowel Dis.* **15,** 1331–6 (2009).

32. Sabino, J. *et al.* Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. *Gut* **65,** 1681–1689 (2016).

33. Ji, S.-G. *et al.* Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease. *Nat. Genet.* (2016). doi:10.1038/ng.3745

34. Navaneethan, U. *et al.* Random biopsies during surveillance colonoscopy increase dysplasia detection in patients with primary sclerosing cholangitis and ulcerative colitis. *J. Crohns. Colitis* **7,** 974–81 (2013).

35. van den Broek, F. J. C. *et al.* Random biopsies taken during colonoscopic surveillance of patients with longstanding ulcerative colitis: low yield and absence of clinical consequences. *Am. J. Gastroenterol.* **109,** 715–22 (2014).

36. Rutter, M. D. *et al.* Most dysplasia in ulcerative colitis is visible at colonoscopy. *Gastrointest. Endosc.* **60,** 334–9 (2004).

37. Iacucci, M. *et al.* Advanced endoscopic techniques in the assessment of inflammatory bowel disease: new technology, new era. *Gut* **68,** 562–572 (2019).

38. Bessissow, T. *et al.* Comparison of Endoscopic Dysplasia Detection Techniques in Patients With Ulcerative Colitis: A Systematic Review and Network Meta-analysis. *Inflamm. Bowel Dis.* **24,** 2518–2526 (2018).

39. Higgins, P. D. R. Miles to Go on the SCENIC Route: Should Chromoendoscopy Become the Standard of Care in IBD Surveillance? *Am. J. Gastroenterol.* **110,** 1035–7 (2015).

40. Marion, J. F. & Sands, B. E. The SCENIC Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease: Praise and Words of Caution. *Gastroenterology* **148,** 462–467 (2015).

41. Fumery, M. *et al.* Incidence, Risk Factors, and Outcomes of Colorectal Cancer in Patients with Ulcerative Colitis with Low-Grade Dysplasia: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* (2016). doi:10.1016/j.cgh.2016.11.025

42. Knudsen, A. B. *et al.* Estimation of Benefits, Burden, and Harms of Colorectal Cancer Screening Strategies. *JAMA* **315,** 2595 (2016).

43. Magro, F. *et al.* Third European Evidence-Based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J. Crohn's Colitis* (2017). doi:10.1093/ecco-jcc/jjx008

44. Farraye, F. A. *et al.* AGA medical position statement on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* **138,** 738–45 (2010).

45. Mooiweer, E., Fidder, H. H., Siersema, P. D., Laheij, R. J. F. & Oldenburg, B. Fecal Calprotectin Testing Can Identify Ineffective Colorectal Cancer Surveillance Procedures in Patients with Longstanding Colitis. *Inflamm. Bowel Dis.* **20,** 1 (2014).

46. Colitis, I. on C. and. HANDLEIDING BEHANDELING IBD. (2015).

47. Andersson, P. & Soderholm, J. D. Surgery in ulcerative colitis: Indication and timing. in *Digestive Diseases* **27,** 335–340 (2009).

48. Cosnes, J., Gower-Rousseau, C., Seksik, P. & Cortot, A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* **140,** 1785–94 (2011).

49. Parks, A. G., Nicholls, R. J. & Belliveau, P. Proctocolectomy with ileal reservoir and anal anastomosis. *Br. J. Surg.* **67,** 533–8 (1980).

50. Derikx, L. A. A. P., Nissen, L. H. C., Smits, L. J. T., Shen, B. & Hoentjen, F. Risk of Neoplasia After Colectomy in Patients With Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* (2015). doi:10.1016/j.cgh.2015.08.042

51. Abdalla, M., Landerholm, K., Andersson, P., Andersson, R. E. & Myrelid, P. Risk of Rectal Cancer After Colectomy for Patients With Ulcerative Colitis: A National Cohort Study. *Clin. Gastroenterol. Hepatol.* **15,** 1055–1060.e2 (2017).

52. Kabir, S. I., Kabir, S. A., Richards, R., Ahmed, J. & MacFie, J. Pathophysiology, clinical presentation and management of diversion colitis: a review of current literature. *Int. J. Surg.* **12,** 1088–92 (2014).

53. Munie, S. *et al.* Fate of the Rectal Stump After Subtotal Colectomy for Ulcerative Colitis in the Era of Ileal Pouch–Anal Anastomosis. *JAMA Surg.* **148,** 408 (2013).

54. Trindade, A. J. *et al.* Devices and methods to improve colonoscopy completion (with videos). *Gastrointest. Endosc.* **87,** 625–634 (2018).

55. Subramanian, V. & Ragunath, K. Advanced endoscopic imaging: a review of commercially available technologies. *Clin. Gastroenterol. Hepatol.* **12,** 368–76.e1 (2014).

56. Kiesslich, R. *et al.* Methylene blue-aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology* **124,** 880–8 (2003).

57. Kiesslich, R. *et al.* Chromoscopy-guided endomicroscopy increases the diagnostic yield of intraepithelial neoplasia in ulcerative colitis. *Gastroenterology* **132,** 874–82 (2007).

58. Freire, P. *et al.* Surveillance in ulcerative colitis: is chromoendoscopy-guided endomicroscopy always better than conventional colonoscopy? A randomized trial. *Inflamm. Bowel Dis.* **20,** 2038–45 (2014).

59. Feuerstein, J. D. *et al.* Detection rates of dysplasia in patients with inflammatory bowel disease using dye-based chromoendoscopy compared with standard- and high-definition white-light colonoscopy: a systematic review and meta-analysis. *Gastrointest. Endosc.* (2019). doi:10.1016/j.gie.2019.04.219

60. Barclay, R. L., Vicari, J. J., Doughty, A. S., Johanson, J. F. & Greenlaw, R. L. Colonoscopic Withdrawal Times and Adenoma Detection during Screening Colonoscopy. *N. Engl. J. Med.* **355,** 2533–2541 (2006).

61. Barclay, R. L., Vicari, J. J. & Greenlaw, R. L. Effect of a time-dependent colonoscopic withdrawal protocol on adenoma detection during screening colonoscopy. *Clin. Gastroenterol. Hepatol.* **6,** 1091–8 (2008).

62. van den Broek, F. *et al.* Narrow-band imaging versus high-definition endoscopy for the diagnosis of neoplasia in ulcerative colitis. *Endoscopy* **43,** 108–115 (2011).

63. Dekker, E. *et al.* Narrow-band imaging compared with conventional colonoscopy for the detection of dysplasia in patients with longstanding ulcerative colitis. *Endoscopy* **39,** 216–221 (2007).

64. Pellisé, M. *et al.* Narrow-band imaging as an alternative to chromoendoscopy for the detection of dysplasia in long-standing inflammatory bowel disease: a prospective, randomized, crossover study. *Gastrointest. Endosc.* **74,** 840–848 (2011).

65. Bisschops, R. *et al.* Chromoendoscopy versus narrow band imaging in UC: a prospective randomised controlled trial. *Gut* **67,** 1087–1094 (2018).

66. Imperatore, N. *et al.* Augmented Endoscopy for Surveillance of Colonic Inflammatory Bowel Disease: Systematic Review With Network Meta-analysis. *J. Crohns. Colitis* **13,** 714–724 (2019).

67. Vleugels, J. L. A. *et al.* Chromoendoscopy versus autofluorescence imaging for neoplasia detection in patients with longstanding ulcerative colitis (FIND-UC): an international, multicentre, randomised controlled trial. *Lancet Gastroenterol. Hepatol.* **3,** 305–316 (2018).

68. The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: November 30 to December 1, 2002. *Gastrointest. Endosc.* **58,** S3-43 (2003).

69. Iacucci, M. *et al.* A multimodal (FACILE) classification for optical diagnosis of inflammatory bowel disease associated neoplasia. *Endoscopy* **51,** 133–141 (2019).

70. Carballal, S. *et al.* Real-life chromoendoscopy for neoplasia detection and characterisation in long-standing IBD. *Gut* gutjnl-2016-312332 (2016). doi:10.1136/gutjnl-2016-312332

71. Wanders, L. K. *et al.* Low interobserver agreement among endoscopists in differentiating dysplastic from non-dysplastic lesions during inflammatory bowel disease colitis surveillance. *Scand. J. Gastroenterol.* **50,** 1011–7 (2015).

72. Shinagawa, T. *et al.* Pine-cone and villi patterns are endoscopic signs suggestive of ulcerative colitis-associated colorectal cancer and dysplasia. *Gastrointest. Endosc.* **89,** 565–575.e3 (2019).

73. Vleugels, J. L. A. *et al.* Diagnostic Accuracy of Endoscopic Trimodal Imaging and Chromoendoscopy for Lesion Characterization in Ulcerative Colitis. *J. Crohn's Colitis* **12,** 1438–1447 (2018).

74. Watanabe, T. *et al.* Comparison of Targeted vs Random Biopsies for Surveillance of Ulcerative Colitis-associated Colorectal Cancer. *Gastroenterology* (2016). doi:10.1053/j.gastro.2016.08.002

75. Moussata, D. *et al.* Are random biopsies still useful for the detection of neoplasia in patients with IBD undergoing surveillance colonoscopy with chromoendoscopy? *Gut* gutjnl-2016-311892 (2017). doi:10.1136/GUTJNL-2016-311892

76. Krugliak Cleveland, N. *et al.* Assessment of peri-polyp biopsy specimens of flat mucosa in patients with inflammatory bowel disease. *Gastrointest. Endosc.* **87,** 1304–1309 (2018).

77. Lahiff, C., Mun Wang, L., Travis, S. P. L. & East, J. E. Diagnostic Yield of Dysplasia in Polyp-adjacent Biopsies for Patients with Inflammatory Bowel Disease: A Cross-sectional Study. *J. Crohn's Colitis* **12,** 670–676 (2018).

78. Wanders, L. K. *et al.* Cancer Risk After Resection of Polypoid Dysplasia in Patients With Longstanding Ulcerative Colitis: A Meta-analysis. *Clin. Gastroenterol. Hepatol.* **12,** 756–764 (2014).

79. Iacopini, F. *et al.* Curative endoscopic submucosal dissection of large nonpolypoid superficial neoplasms in ulcerative colitis (with videos). *Gastrointest. Endosc.* **82,** 734–8 (2015).

80. Suzuki, N., Toyonaga, T. & East, J. E. Endoscopic submucosal dissection of colitis-related dysplasia. *Endoscopy* (2017). doi:10.1055/s-0043-114410

81. Kinoshita, S. *et al.* The role of colorectal endoscopic submucosal dissection in patients with ulcerative colitis. *Gastrointest. Endosc.* **87,** 1079–1084 (2018).

82. Shigeyasu, K., Toden, S., Zumwalt, T. J., Okugawa, Y. & Goel, A. Emerging Role of MicroRNAs as Liquid Biopsy Biomarkers in Gastrointestinal Cancers. *Clin. Cancer Res.* **23,** 2391–2399 (2017).

83. Kanaan, Z. *et al.* A plasma microRNA panel for detection of colorectal adenomas: a step toward more precise screening for colorectal cancer. *Ann. Surg.* **258,** 400–8 (2013).

84. Carter, J. V. *et al.* A Highly Predictive Model for Diagnosis of Colorectal Neoplasms Using Plasma MicroRNA. *Ann. Surg.* **264,** 575–584 (2016).

85. Johnson, D. H. *et al.* DNA Methylation and Mutation of Small Colonic Neoplasms in Ulcerative Colitis and Crohn's Colitis: Implications for Surveillance. *Inflamm. Bowel Dis.* (2016). doi:10.1097/MIB.0000000000000795

86. Kisiel, J. B. *et al.* Analysis of DNA Methylation at Specific Loci in Stool Samples Detects Colorectal Cancer and High-Grade Dysplasia in Patients With Inflammatory Bowel Disease. *Clin. Gastroenterol. Hepatol.* **17,** 914–921.e5 (2019).

87. Feng, Y. *et al.* MicroRNA–449a is a potential predictor of colitis–associated colorectal cancer progression. *Oncol. Rep.* **40,** 1684–1694 (2018).

88. Patel, N. R., McPhail, M. J., Shariff, M. I., Keun, H. C. & Taylor-Robinson, S. D. Biofluid metabonomics using ^{1}H NMR spectroscopy: the road to biomarker discovery in gastroenterology and hepatology. *Expert Rev. Gastroenterol. Hepatol.* **6,** 239–251 (2012).

89. Viennois, E., Zhao, Y. & Merlin, D. Biomarkers of Inflammatory Bowel Disease: From Classical Laboratory Tools to Personalized Medicine. *Inflamm. Bowel Dis.* **21,** 2467–74 (2015).

90. van Maaren, M. C. *et al.* Validation of the online prediction tool PREDICT v. 2.0 in the Dutch breast cancer population. *Eur. J. Cancer* **86,** 364–372 (2017).

91. Choi, C.-H. R. *et al.* Cumulative burden of inflammation predicts colorectal neoplasia risk in ulcerative colitis: a large single-centre study. *Gut* **68,** 414–422 (2019).

92. Parian, A. M. & Lazarev, M. G. Serrated Colorectal Lesions in Patients With Inflammatory Bowel Disease. *Gastroenterol. Hepatol. (N. Y).* **14,** 19–25 (2018).

93. Bronner, M. P. *et al.* Array-based comparative genomic hybridization in ulcerative colitis neoplasia: single non-dysplastic biopsies distinguish progressors from non-progressors. *Mod. Pathol.* **23,** 1624–1633 (2010).

94. Beggs, A. D. *et al.* Discovery and Validation of Methylation Biomarkers for Ulcerative Colitis Associated Neoplasia. *Inflamm. Bowel Dis.* **24,** 1503–1509 (2018).

95. Garrity-Park, M. M., Loftus, E. V., Bryant, S. C. & Smyrk, T. C. A Biomarker Panel to Detect Synchronous Neoplasm in Non-neoplastic Surveillance Biopsies from Patients with Ulcerative Colitis. *Inflamm. Bowel Dis.* **22,** 1568–1574 (2016).

96. Xue, X. *et al.* Quantitative proteomics identifies STEAP4 as a critical regulator of mitochondrial dysfunction linking inflammation and colon cancer. *Proc. Natl. Acad. Sci.* **114,** E9608–E9617 (2017).

97. Brentnall, T. A. *et al.* Proteins That Underlie Neoplastic Progression of Ulcerative Colitis. *Proteomics. Clin. Appl.* **3,** 1326 (2009).

98. Imperiale, T. F. *et al.* Multitarget stool DNA testing for colorectal-cancer screening. *N. Engl. J. Med.* **370,** 1287–97 (2014).

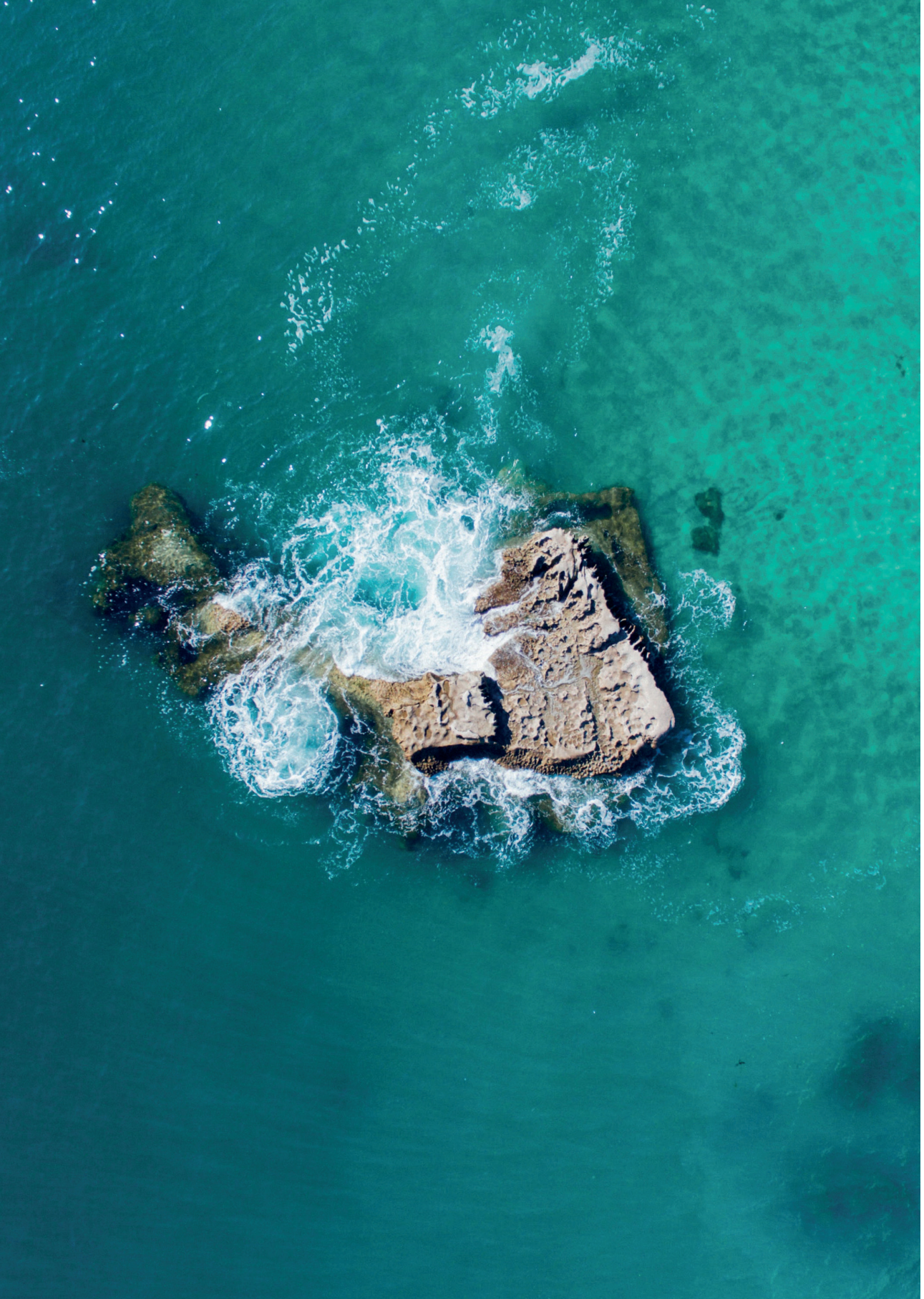
99. Wang, Y. *et al.* Prognostic Potential of Circulating Tumor DNA Measurement in Postoperative Surveillance of Nonmetastatic Colorectal Cancer. *JAMA Oncol.* (2019). doi:10.1001/jamaoncol.2019.0512

100. Petit, J. *et al.* Cell-Free DNA as a Diagnostic Blood-Based Biomarker for Colorectal Cancer: A Systematic Review. *J. Surg. Res.* **236,** 184–197 (2019).

101. Ananthakrishnan, A. N. *et al.* Serum inflammatory markers and risk of colorectal cancer in patients with inflammatory bowel diseases. *Clin. Gastroenterol. Hepatol.* **12,** 1342–8.e1 (2014).

102.Lumachi, F., Marino, F., Orlando, R., Chiara, G. B. & Basso, S. M. M. Simultaneous multianalyte immunoassay measurement of five serum tumor markers in the detection of colorectal cancer. *Anticancer Res.* **32,** 985–8 (2012).

103.Schicho, R. *et al.* Quantitative metabolomic profiling of serum, plasma, and urine by (1)H NMR spectroscopy discriminates between patients with inflammatory bowel disease and healthy individuals. *J. Proteome Res.* **11,** 3344–57 (2012).



APPENDIX

ACKNOWLEDGEMENTS (DANKWOORD)

ACKNOWLEDGEMENTS / DANKWOORD

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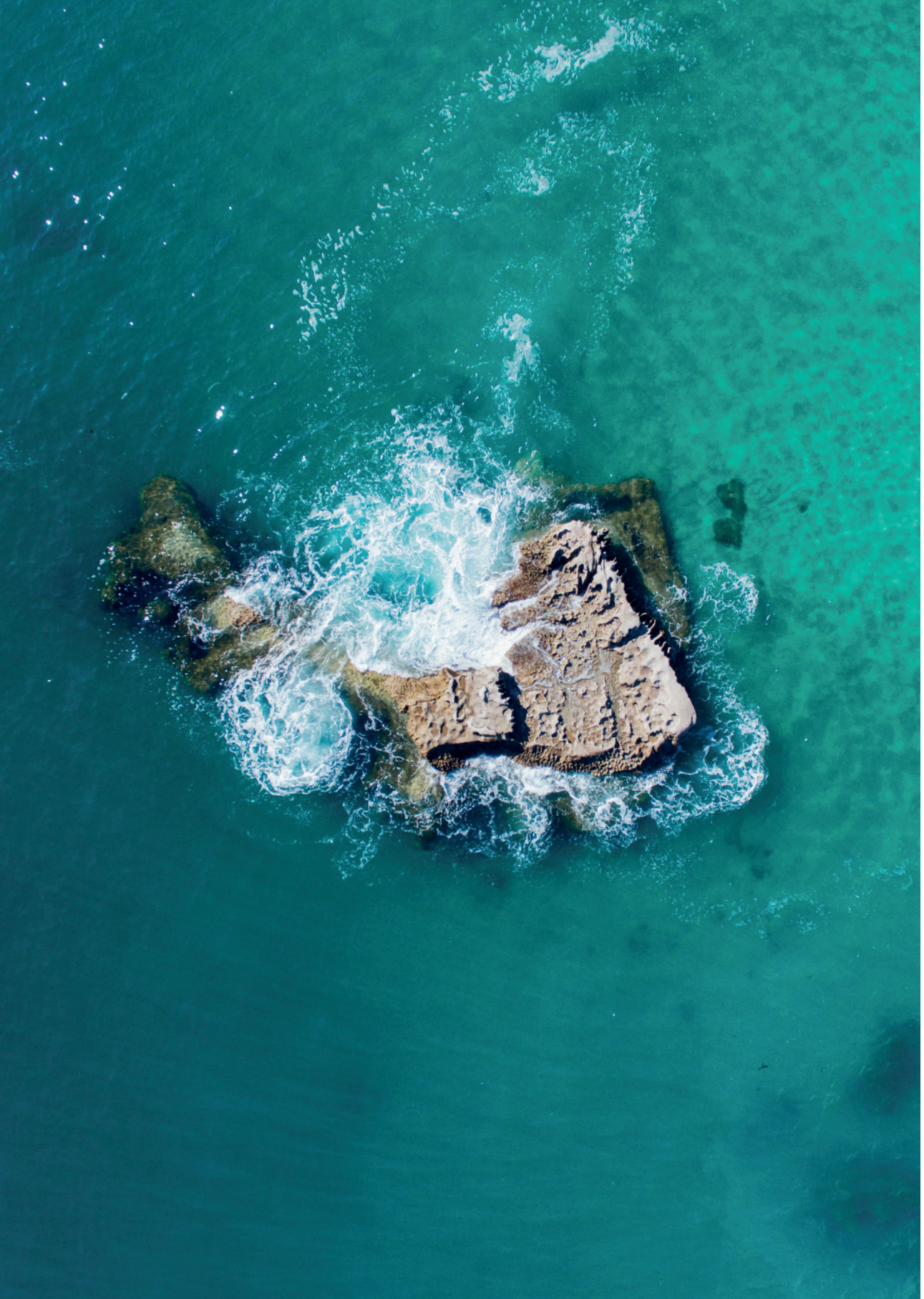

ABOUT THE AUTHOR

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Joren René ten Hove was born in Utrecht, the Netherlands in 1989. He attended Christelijk Gymnasium Utrecht and after graduating, started studying Medicine at Utrecht University in 2008.

As a medical student, his interest in scientific research was sparked by doing a research project under supervision of Usama Ahmed Ali, which was focused on reporting of surgical clinical trials. Later, during his medical internships, he started focusing on gastroenterology. In his final year of medical school, he started a research internship on submucosal lesions of the upper gastrointestinal tract under supervision of dr. Leon Moons. Meanwhile, he completed his clinical rotations in hospitals in and around Utrecht and the Kasturba Hospital in Manipal, India.

After completing his final clinical and research internships at the department of Gastroenterology & Hepatology of the UMC Utrecht, he obtained his medical degree in 2015. Subsequently, he started his PhD research project at that same department under supervision of prof. dr. P.D. Siersema and dr. B. Oldenburg.

Following his years as a full time researcher, he started as a gastroenterologist-in-training in 2018. Joren is currently working at the Meander Medisch Centrum in Amersfoort, as part of the first part of his residency program in Internal Medicine (supervisor: dr. R.J. Bosma and R. Fijnheer).