Biomarkers for inflammation and surveillance strategies in inflammatory bowel disease

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Thesis with summary in Dutch, University of Utrecht

The printing of this thesis was financially supported by:
Afdeling Maag-, Darm-, Leverziekten UMC Utrecht, Ferring BV, Nederlandse Vereniging voor Gastroenterologie, Tramedico BV

Cover and Layout:	Frans van Ditzhuijzen
Printing:	Ridderprint BV, Ridderkerk, the Netherlands
ISBN:	978-90-5335-537-2

Biomarkers for inflammation and surveillance strategies in inflammatory bowel disease

Biomarkers voor ontsteking en strategieën voor surveillance in inflammatoire darmziekten
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op 2 december 2014 des middags te 2.30 uur

door

Erik Mooiweer
geboren op 2 juli 1985 te Nieuwegein

Promotor:	Prof. dr. P.D. Siersema
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Chapter 1

General Introduction

Inflammatory bowel disease

Ulcerative colitis (UC) and Crohn's disease (CD) are the two main subtypes of inflammatory bowel disease (IBD). IBD is characterized by episodes of inflammation, which is restricted to the colon in UC whereas the entire gastrointestinal tract may be affected in patients with CD. Although the exact cause of IBD is still unknown, several genetic mutations, a shift in microbiota composition and a change in the innate immune system combined with largely unknown environmental triggers are all thought to contribute to the development of IBD. [1, 2]

The age of onset of IBD is generally around the second and third decade, affecting men and women equally. In the Netherlands, the incidence of UC is 10.0 per 100.000 inhabitants and 6.9 per 100.000 inhabitants for CD, with a combined estimated prevalence of 326 IBD patients per 100.000 inhabitants.[3]

IBD and colorectal cancer

It is commonly accepted that colonic inflammatory bowel disease (IBD) is associated with an increased risk of colorectal cancer (CRC). It has been postulated that the ongoing inflammation of the colonic mucosa in IBD triggers the inflammation – dysplasia – carcinoma sequence through several genetic alterations.[4] An often cited meta-analysis reported a cumulative incidence as high as 2%, 10% and 18% after 10, 20 and 30 years of disease duration.[5] Recent studies have shown a considerably lower CRC incidence of 5% after 30 years of disease duration however, although this was still significantly higher than the background population.[6] One important explanation for the large heterogeneity among the studies on the incidence of CRC among IBD patients is the fact that the CRC incidence is higher in studies including data from referral centers than is reported in population based studies. In **chapter 2** we looked at patient characteristics potentially explaining these differences, by comparing IBD patients diagnosed with CRC in referral centers to those diagnosed with CRC in general hospitals.

Endoscopic surveillance

The goal of endoscopic surveillance is to detect and treat dysplasia or asymptomatic CRC and thereby reducing the risk of CRC-related mortality. Despite the fact that numerous national and international guidelines recommend endoscopic surveillance in IBD patients, there are no randomized trials comparing surveillance to no surveillance that provide evidence that this strategy is effective. There is some circumstantial evidence that surveillance is indeed effective, as three retrospective studies showed that CRC is detected at an earlier stage among patients that underwent surveillance and that this is associated with better overall survival.[7-9]

As surveillance is aimed at detecting dysplasia and early stage CRC, surveillance can be considered ineffective when advanced stage CRC is diagnosed despite endoscopic surveillance

performed in accordance with international guidelines. In **chapter 3** we assessed the prevalence of so-called interval CRCs among a large cohort of IBD patients enrolled in a surveillance program, in order to gain further evidence as to whether the strategy of regular endoscopic surveillance is effective.

Surveillance guidelines

There are several well-established risk factors for IBD-associated CRC. Since inflammation is the driving force behind the development of CRC in IBD patients, the risk of CRC correlates with the amount of colonic surface involved in the inflammatory process, severity of inflammation and disease duration.[10, 11] Furthermore, a positive family history of CRC and a concomitant diagnosis of primary sclerosing cholangitis increase the risk of CRC.[12] These risk factors were incorporated in the first surveillance guidelines of the American Gastroenterological Association (AGA) and the British Society of Gastroenterology (BSG), on which most other national guidelines are based.[13, 14] Both guidelines state that surveillance should be initiated after a disease duration of 8-10 years in UC patients with at least left-sided colitis and in CD patients with involvement of at least 50% of the colon.

A more recent study by Rutter et al. showed that endoscopic features encountered during surveillance can also help determining the CRC risk, such as the presence of post-inflammatory polyps, strictures and severe inflammation.[15] In 2010 the updated BSG guideline was published which adopted an algorithm based on established risk factors to stratify patients in a high, intermediate and low risk group with corresponding surveillance intervals of 1, 3 and 5 years.[16] Although the AGA guideline was updated as well, only patients with a concomitant PSC diagnosis were selected as high-risk patients with a corresponding surveillance interval of one year and a recommended interval between 1 and 3 years for all remaining patients.[16, 17] Triggered by these differences in risk stratification and surveillance intervals, we compared the colonoscopic workload and neoplasia yield between both guidelines using a large retrospective cohort of IBD patients undergoing surveillance in **chapter 4**.

Detection of dysplasia in IBD patients

The effectiveness of surveillance in preventing the development of CRC critically depends on the ability to identify dysplasia, which can be subdivided in low-grade dysplasia (LGD) and high-grade dysplasia (HGD). Endoscopically, visible raised dysplastic lesions are subdivided based on their appearance into adenoma-like mass (ALM) or dysplasia-associated lesion or mass (DALM). ALM constitutes all raised lesions resembling sporadic adenomas also observed in patients without IBD, whereas the term DALM refers to all other dysplastic lesions i.e. irregular, diffuse masses or plaques. If a lesion is classified as a DALM, there is a high risk of

synchronous and metachronous CRC and surveillance guidelines therefore recommend colectomy.[18] Although some studies show that the risk of developing CRC is low in patients with an ALM provided that it can be resected in total, it is unclear whether an ALM in a background of colonic inflammation is associated with an increased risk of CRC compared to IBD patients without this lesion.[19-22] In **chapter 5** we therefore compared the risk of developing either HGD or CRC among three groups of patients, i.e. IBD patients with an ALM, IBD patients without an ALM and non-IBD patients with a sporadic adenoma.

In addition to ALMs and DALMs, a variety of non-dysplastic lesions can be encountered during surveillance as well, i.e. scars, post-inflammatory polyps and hyperplastic polyps. The endoscopic differentiation between all these types of lesions (non-dysplastic, ALM and DALM) has important clinical consequences, since a DALM is considered to be an indication for prophylactic colectomy whereas all other lesions are preferably managed endoscopically. **Chapter 6** was aimed at investigating the accuracy and inter-observer agreement among expert and non-expert endoscopists in differentiating between dysplastic and non-dysplastic lesions and between ALM and DALM using a digital questionnaire containing a variety of lesions encountered during IBD surveillance.

Performing surveillance in patients with IBD implies complete colonic inspection and taking biopsies of lesions suspected of containing neoplasia, as well as taking four quadrant random biopsies from normal appearing mucosa every 10 cm throughout the colon. The rationale for these random biopsies is that dysplasia can be present, even in normal appearing colonic mucosa.[23] The diagnostic yield of these random biopsies is disappointingly low however, as only five cases of dysplasia were detected in a total of 11.772 random biopsies sampled.[24] In addition, it seems that nearly all cases of dysplasia are macroscopically visible, casting further doubt on the clinical value of taking random biopsies.[25, 26] In an effort to find new techniques to enhance dysplasia detection, two randomized controlled trials and two studies with a back-to-back study design have demonstrated an increased dysplasia yield when using chromoendoscopy.[27-30] This technique comprises the coloring of the entire colonic mucosa with either indigo carmine or methylene blue, which delineates the borders of subtle, flat dysplastic lesions thereby improving dysplasia detection. Because chromoendoscopy is presently considered the preferred surveillance method it has recently been implemented in three university medical centers in the Netherlands. In **chapter 7**, we compared the dysplasia yield of these chromoendoscopies to the surveillance colonoscopies performed with white light endoscopy and random biopsies in a retrospective case-control design. This study was aimed at investigating whether chromoendoscopy could indeed increase the dysplasia detection rate in clinical practice.

Biomarkers for inflammation

The clinical course of IBD is unpredictable and characterized by episodes of flares causing symptoms such as abdominal pain and (bloody) diarrhea and of remission. Traditionally, treatment of both CD and UC patients was aimed at achieving symptom relief and clinical remission. Since the emergence of newer treatment modalities such as anti-TNFa, more stringent treatment goals became achievable such as sustained steroid-free remission and endoscopic remission (mucosal healing). It has recently become apparent that achieving sustained mucosal healing in IBD patients is associated with a significant reduction in the number of hospitalizations and surgeries.[31] Therefore, clinical remission combined with mucosal healing is now considered as the ultimate treatment goal in patients with IBD.[32] This of course means that the inflammatory activity of the bowel needs to be monitored in order to evaluate response to the initiated treatment. This is challenging however, since clinical symptoms are not reliable proxies for inflammation in IBD patients.[33, 34] The only reliable method to establish the presence of mucosal healing is colonoscopy, which is burdensome for patients, associated with high costs and a risk of complications. Therefore, there is a need for an easily obtainable non-invasive marker for inflammation to assess treatment outcome. Multiple studies have investigated both clinical and serological scoring systems and their correlation with endoscopic disease activity, of which fecal calprotectin seems the most promising marker. Calprotectin is a protein present in the cytosol of neutrophilic granulocytes, which can be measured in small stool samples and remains stable at room temperature for up to seven days. Granulocytes release calprotectin in case of cell activation or cell death and are detectable in serum, saliva, urine and stool.[35] Roseth et al. was the first to measure calprotectin levels in stool samples and reported higher levels among IBD patients compared to healthy controls.[36] Since then, several studies have shown that a good correlation exists between calprotectin levels in stool and inflammatory activity during ileocolonoscopy in both CD and UC patients, with correlation coeficients ranging between 0.42 and 0.79 in CD and between 0.51 and 0.83 in UC.[37-41] Although fecal calprotectin is the best available non-invasive marker to date and now widely used in clinical practice, the diagnostic accuracy is still not high enough to solely base treatment decisions on this parameter. In **chapter 8**, we therefore compared the diagnostic accuracy of fecal calprotectin to another fecal marker, haemoglobin, to find either a new marker with higher diagnostic accuracy or to improve the diagnostic accuracy by combining both markers.

When analyzing the data of chapter 8, we were intrigued by the fact that some patients had elevated calprotectin levels, despite complete mucosal healing during colonoscopy. In **chapter 9** we investigated two hypotheses associated with these elevated calprotectin levels. Studies have shown that histological inflammation can be more extensive than endoscopically appreciated.[42] This histological inflammation may also explain the elevated calprotectin levels,

despite mucosal healing during colonoscopy. In addition, this ongoing histological inflammation may be associated with an increased risk of relapse during follow-up, similar to the known increased risk observed for endoscopically visible inflammatory activity.

Using calprotectin to optimize surveillance

As mentioned previously, the detection of dysplasia during surveillance colonoscopy in IBD patients can be challenging due to the frequently encountered flat architecture of the colitis-associated dysplastic lesions. Surveillance procedures should therefore be performed only when the bowel is adequately cleansed, with high definition endoscopes and taking sufficient withdrawal time. In addition, it is important that the colon is free of inflammation, as this renders identification of dysplasia even more difficult for both the endoscopist and pathologist. In **chapter 10** we investigated whether the fecal marker calprotectin can identify patients with moderate or severe inflammation, in whom the surveillance procedure is probably ineffective.

Discussion and summary

In **chapter 11**, the results are discussed including their implications for clinical practice and future research.

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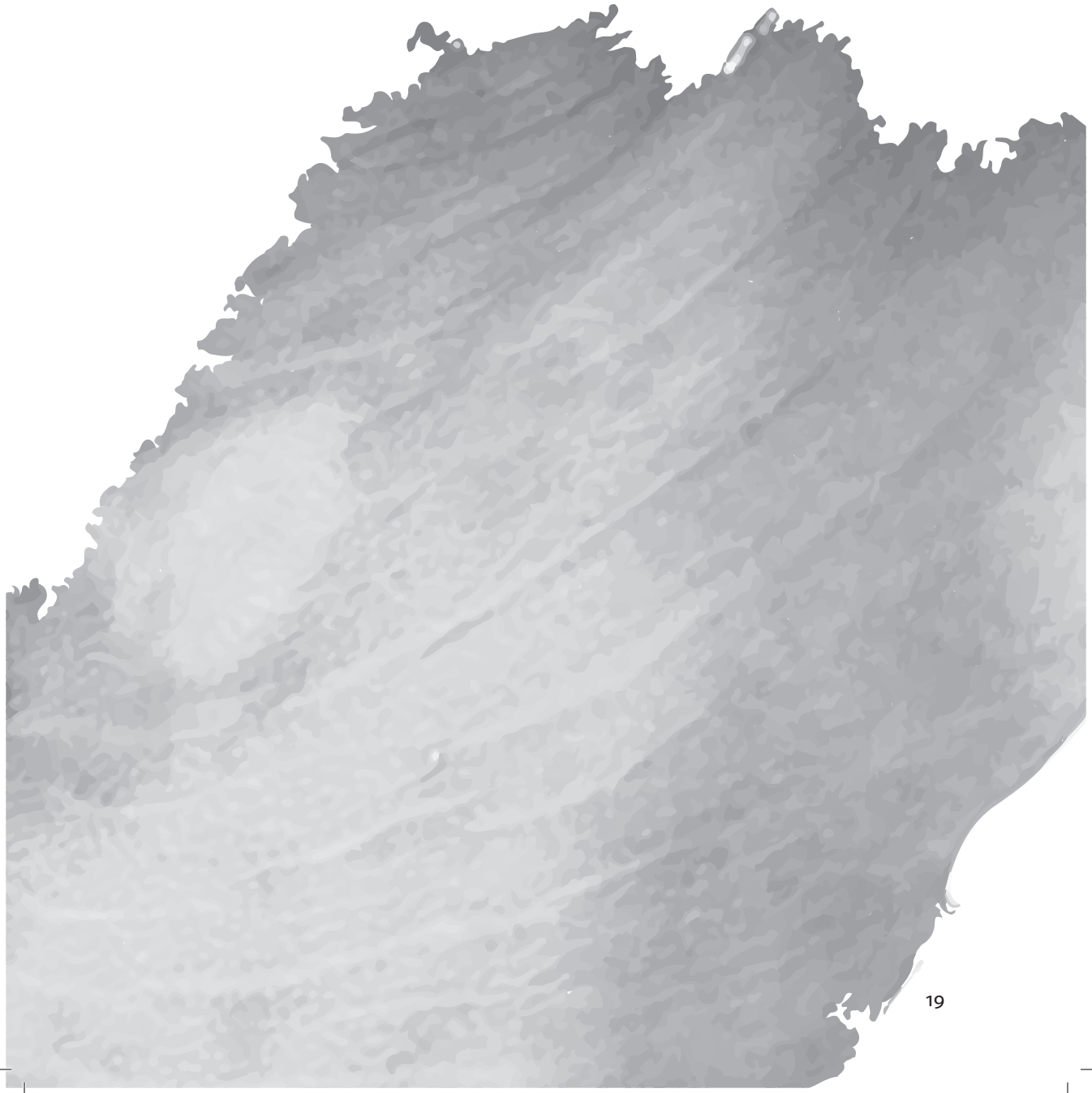
Part I:

Surveillance for colorectal cancer in inflammatory bowel disease



Chapter 2

Disease severity does not affect the interval between IBD diagnosis and the development of CRC: Results from two large, Dutch case series

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Published in Journal of Crohn's and colitis

ABSTRACT

Background

The increased risk of colorectal cancer (CRC) in patients with inflammatory bowel disease (IBD) is well established. The incidence of IBD-related CRC however, differs markedly between cohorts from referral centers and population-based studies. In the present study we aimed to identify characteristics potentially explaining these differences in two cohorts of patients with IBD-related CRC.

Methods

PALGA, a nationwide pathology network and registry in The Netherlands, was used to search for patients with IBD-associated CRC between 1990 and 2006. Patients from 7 referral hospitals and 78 general hospitals were included. Demographic and disease specific parameters were collected retrospectively using patient charts.

Results

A total of 281 patients with IBD-associated CRC were identified. Patients from referral hospitals had a lower median age at IBD diagnosis (26 years vs. 28 years ($p=0.02$)), while having more IBD-relapses before CRC diagnosis than patients from general hospitals (3.8 vs. 1.5 ($p<0.01$)). In patients from referral hospitals, CRC was diagnosed at a younger age (47 years vs. 51 years ($p=0.01$)). However, the median interval between IBD diagnosis and diagnosis of CRC was similar in both cohorts (19 years in referral hospitals vs. 17 years in general hospitals ($p=0.13$)).

Conclusions

IBD Patients diagnosed with CRC treated in referral hospitals in The Netherlands are younger at both the diagnosis of IBD and CRC than IBD patients with CRC treated in general hospitals. Although patients from referral centers appeared to have a more severe course of IBD, the interval between IBD and CRC diagnosis was similar.

INTRODUCTION

Longstanding inflammatory bowel disease (IBD) increases the risk of colorectal carcinoma (CRC), although the risk may be lower than previously perceived. A frequently cited meta-analysis reported cumulative incidence rates for patients with ulcerative colitis (UC) up to 18% after 30 years of disease [1]. Patients with Crohns' colitis (CD) seem to carry a slightly lower risk of CRC than UC patients [2,3]. Although these data are widely acknowledged and have been used to design surveillance guidelines for patients with longstanding IBD [4,5], there is an ongoing debate about the magnitude of the risk of CRC in patients with colitis. Remarkably, the CRC risks derived from population-based studies are consistently lower than those from referral center-based studies [6-9]. This discrepancy has been attributed to differences in disease severity. The more severe IBD-phenotype in patients cared for in referral hospitals is thought to result in a higher CRC risk in the long run [10]. Whether differences in treatment or demographic parameters play a role is presently unknown.

The aim of the present study was to compare two large cohorts of IBD patients with CRC with regard to demographic and disease specific characteristics. We compared a cohort from referral hospitals (RH's) with a cohort from general hospitals (GH's) and hypothesized that IBD patients from RH's had more extensive and severe disease, resulting in an accelerated inflammation-dysplasia-carcinoma sequence with subsequently early development of IBD-related CRC as compared to IBD patients from GH's.

PATIENTS AND METHODS

Patients

PALGA, a nationwide histopathology database in the Netherlands, was used to identify patients with IBD and a subsequent diagnosis of CRC [11]. We identified all IBD patients diagnosed with CRC in 78 general hospitals and 7 referral hospitals in the period from 1990 until 2006. To minimize interference with sporadic CRC, all patients with CRC diagnosed above the age of 65 were excluded. Patients were recruited from referral hospitals (RH's) and general hospitals (GH's). Both cohorts have been described before [12,13]. Patients diagnosed with CRC before or within 12 months after the diagnosis of IBD were excluded.

Data collection

Data were collected by two researchers using identical data collection forms and employing the same definitions. Variables and definitions have been reported in detail in previous reports

(12;13). In brief, the maximum histological and endoscopic extent and severity of inflammation (scored as mild, moderate or severe) were obtained from all endoscopy and histopathology reports prior to CRC diagnosis. In UC and indeterminate colitis disease extent was considered left-sided if the most proximal point of inflammation was distal to the splenic flexure. Inflammation extending proximally to the splenic flexure was considered extensive disease. In CD, extensive disease was defined as involvement of more than 50% of the colonic mucosa. The number of exacerbations was defined as all documented endoscopic flares or occurrence of IBD-related symptoms requiring a change in medication, surgery or hospitalization.

Statistical analysis

Categorical variables were analyzed using the Chi-square test. Continuous variables were analyzed with Student's t-test or Mann-Whitney U-test depending on data distribution. Kaplan-Meier survival analysis was used to calculate CRC-related mortality and the log rank test was used to compare CRC-related mortality between GH patients and RH patients. A two sided p-value <0.05 was considered significant. SPSS version 15 for Windows was used to perform all statistical analyses.

Ethical considerations

This study was carried out with the approval of and in accordance with the privacy and ethical guidelines of the privacy committee of PALGA and in accordance with the ethical guidelines of the research committee of both institutions.

RESULTS

Patients

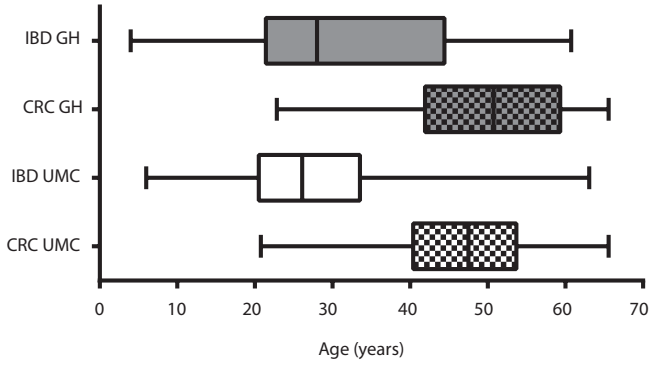
After the PALGA search and review of the patient files, a diagnosis of CRC after a diagnosis of IBD was confirmed in 346 patients. A total of 65 patients were excluded. Reasons for exclusion were the simultaneous diagnosis of CRC and IBD (31 patients), a CRC diagnosis within 12 months after IBD diagnosis (13 patients), CRC diagnosis at an age of 65 years or older (18 patients) and unknown date of IBD diagnosis (3 patients). In total, 281 patients were included for further analysis. One-hundred and twenty-one (43%) patients were treated in RH's and 160 (57%) patients were treated in GH's (from 78 of 93 GH's present in The Netherlands). Men were more frequently affected by CRC than women (61% compared to 39%). Median age at diagnosis of IBD was 27 years (IQR 18.5 – 36.1) Median age at diagnosis of CRC was 50 years (range 20 – 65 years).

In total, 177 (63%) patients were diagnosed with UC, 102 (36%) with CD and 2 (1%) with indeterminate colitis. Median age at diagnosis of CD was 26 years (IQR 18 - 33) as compared to 28 years (IQR 18 - 37) in UC patients (p=0.06). Median age at diagnosis of CRC was similar in CD patients (49 years, IQR 40 - 57) and UC patients (50 years, IQR 41 – 58, p=0.58). However, the median interval between IBD and CRC diagnosis was shorter in UC patients (17 years, IQR 12.2 – 27.8) than in CD patients (20 years, IQR 10.4 – 24.4, p=0.02).

Comparison between RH and GH patients

Median age at CRC diagnosis was lower in RH patients than in GH patients (47 years vs. 51 years (p=0.01)). Moreover, patients in RH's were diagnosed with IBD at a younger age (26 vs. 28 years (p=0.02)). The interval between IBD diagnosis and diagnosis of CRC was similar in both cohorts (19 years in RH's vs.17 years in GH's (p=0.13)) (Figure 1).

Figure 1: Box Whisker plot displaying age at IBD diagnosis and age at colorectal cancer (CRC) diagnosis in patients from Referral hospitals (RH) and General hospitals (GH).



Clinical parameters

Data regarding the endoscopic and histologic extent of inflammation before CRC diagnosis was available in 209 (74%) and 211 (75%) patients respectively. Extensive disease in both UC and CD was encountered more frequently in RH's, but differences were only significant when using the histologic definition of extensive disease (p<0.01) (Table 1). Endoscopic or histologic classification of the severity of inflammation was available in 256 (91%) and 261 (93%) patients respectively. Severe inflammation during endoscopy was present in 68 (59%) RH patients and 60 (43%) GH patients (p=0.01). Histologic diagnosis of severe inflammation was found in 82 (71%) RH patients and 69 (48%) GH patients (p<0.01). Mean number of exacerbations was 3.8 and 1.5 for RH patients and GH patients (p<0.01).

Data regarding medication use was unavailable in 14 patients. The number of patients treated

with 5-ASA, thiopurines, corticosteroids and anti-TNF was higher in RH patients compared to GH patients (Table 1). Tumor location, tumor stage and the type of surgical resection were similar in both cohorts. (Table 2).

When the cohorts of GH patients and the RH patients were combined, the presence of endoscopic extensive disease, endoscopic severe inflammation, concomitant diagnosis of PSC and number of exacerbations did not result in a significantly lower interval between IBD and CRC diagnosis (all p>0.05 cox regression).

Table 2: comparison of tumor characteristics of IBD patients diagnosed with CRC in general hospitals and referral hospitals.

	Referral hospitals (n=121)	**General hospitals (n=160)**	**P-value**
Tumor location			0.10
Rectum	32/107 (30%)	42/146 (29%)	
Sigmoid	27/107 (25%)	34/146 (23%)	
Descendens	7/107 (7%)	16/146 (11%)	
Transversum	15/107 (14%)	21/146 (14%)	
Ascendens	18/107 (17%)	11/146 (8%)	
Coecum	8/107 (7%)	22/146 (15%)	
Tumor stage			0.26
Dukes A/B	59/105 (56%)	75/153 (49%)	
Dukes C/D	46/105 (43%)	78/153 (51%)	
Type of surgical resection			0.28
Segmental resection	26/114 (23%)	43/128 (34%)	
Subtotal colectomy	32/114 (28%)	24/128 (19%)	
Total colectomy	38/114 (33%)	39/128 (30%)	
Proctectomy	14/114 (12%)	16/128 (12%)	
No surgical resection	4/114 (4%)	6/128 (5%)	

Table 1: comparison of baseline characteristics of patients diagnosed with IBD related CRC in Referral hospitals and General hospitals.

	Referral hospitals (n=121)	**General hospitals (n=160)**	**P-value**
Male Gender	71/121 (60%)	100/160 (62%)	0.62
Type of IBD			
Crohn's disease	47/121 (39%)	55/160 (34%)	0.35
Ulcerative colitis	74/121 (61%)	104/160 (65%)	0.35
Indeterminate colitis	0/121 (0%)	1/160 (1%)	NA
Median age at IBD diagnosis (yr, IQR)	26 (20-33)	28 (17-39)	0.02
Median age at CRC diagnosis(yr, IQR)	48 (41 – 54)	51 (42-60)	0.01
Median interval between diagnosis of IBD and CRC (yr, IQR)	19 (13-26)	17 (10-25)	0.13
Positive family history of CRC	6/45 (14%)	11/78 (17%)	0.91
Primary sclerosing cholangitis	17/120 (14%)	17/146 (12%)	0.54
Extensive disease			
Endoscopic	79/107 (72%)	72/102 (67%)	0.60
Histologic	99/112 (86%)	70/99 (70%)	<0.01
Severe inflammation			
Endoscopic	65/107 (59%)	51/117 (43%)	0.01
Histologic	78/108 (71%)	58/122 (48%)	<0.01
Presence of post-inflammatory polyps	50/84 (60%)	69/130 (53%)	0.35
Medication use			
5-ASA	101/114 (89%)	114/153 (75%)	<0.01
Thiopurines	39/114 (34%)	37/153 (24%)	0.05
Corticosteroids	82/114 (72%)	80/153 (52%)	<0.01
Anti-TNF	10/114 (9%)	2/153 (1%)	<0.01

CRC, colorectal cancer IQR, inter quartile range

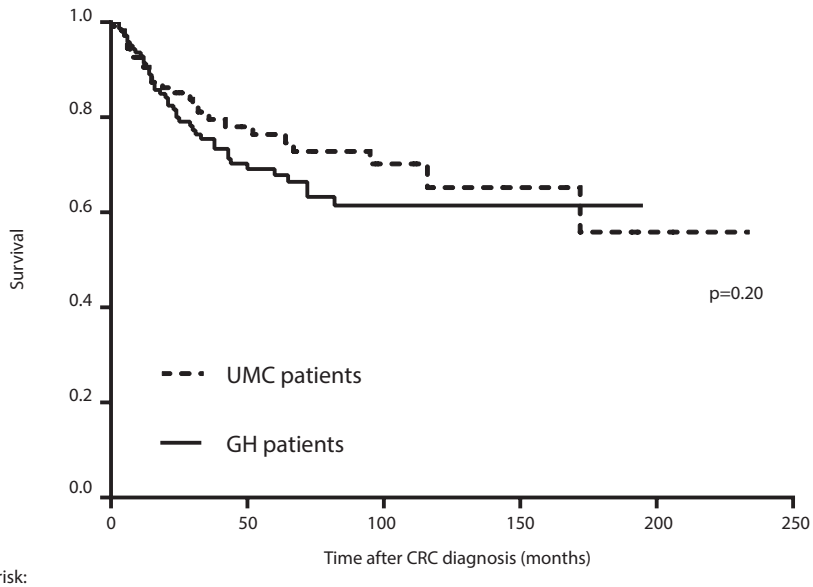
Surveillance

In total, 40 (14%) patients were enrolled in a surveillance program before the diagnosis of CRC. The percentage of patients enrolled in a surveillance program was not different between the two cohorts (12% in RH's vs 16% in GH's (p=0.58)).

Survival

Follow-up data after CRC diagnosis until the end of the study period or death was available in 223 (79%) patients, whereas58 (21%) patients were lost to follow-up. In the total study population, 83 patients died, of whom 71 patients died because of (metastatic) CRC. Kaplan-Meier analysis showed no difference in CRC related mortality between RH and GH patients (p=0.20) (Figure 2).

Figure 2: Kaplan-Meier curve comparing survival in patients from referral hospitals (RH) and general hospitals (GH) after diagnosis of colorectal cancer (CRC) (log rank test P = 0.20).



Patients at risk:

RH patients	120	50	23	10	3	0
GH patients	161	61	26	11	1	1

DISCUSSION

In this large retrospective nationwide cohort of patients with IBD related CRC, we confirmed that IBD patients from RH's represent a subgroup with a more severe IBD-phenotype. Since severity and extent of inflammation are considered important risk factors for the development of CRC in IBD patients, we hypothesized that a more severe IBD-phenotype would result in a shorter interval between a diagnosis of IBD and developing CRC. This assumption could not be confirmed in the present study. IBD Patients from RH's had a more severe and extensive disease than IBD patients from GH's, but the interval between IBD and CRC diagnosis was similar. Moreover, after combining the two cohorts, presence of extensive or severe inflammation or a concomitant diagnosis of PSC were not associated with a shorter interval between IBD and CRC diagnosis. Apparently, severity of inflammation is not clearly setting the pace of the inflammation-carcinoma sequence in IBD-patients. This finding conflicts with the widespread concept that disease activity in individual patients in the past should be taken into account when determining the colonoscopy intervals for CRC surveillance programs as was proposed in the recently updated BSG guidelines for surveillance in IBD patients [4]. Previous studies focusing on the interval between IBD and CRC diagnosis reported that a higher age at IBD diagnosis was associated with a shorter interval between IBD and CRC diagnosis and that extent of inflammation did not influence the interval between IBD and CRC diagnosis [14-16]. Together, this suggests that higher age itself is a major risk factor for CRC in IBD, and that disease duration is of less importance. This is in contrast with the widely accepted view that the risk of CRC in IBD patients increases with disease duration [1,17].

We previously reported risk factors for IBD-associated CRC in a case-control study based on the data from GH patients [13]. Although we did not include a matched control cohort in the current study, our results are in line with previous studies. First, the male predominance is in concordance with the study of Soderlund et al., who reported higher relative and absolute risks of CRC in male IBD patients than in female IBD patients [18]. Secondly, a high prevalence of PSC of 12% was found in patients from RH's as well as GH's. This is markedly higher than the 2% prevalence of PSC which has previously been reported in large population-based IBD cohorts [19,20]. This underscores the widely acknowledged fact that PSC is an independent risk factor for CRC development in IBD patients [21-23]. Thirdly, a large percentage of patients displayed a severe IBD phenotype before a diagnosis of CRC and were found to have post-inflammatory polyps (56%), which is in line with previously reported risk factors [24].

Remarkably, only 14% of patients received one or more surveillance colonoscopies prior to CRC diagnosis. This is partly due to the fact that 64 patients did not have a strict indication for surveillance because CRC developed within 8 years of IBD diagnosis or disease extent was limited

to proctitis. After exclusion of these patients, 18% received surveillance prior to CRC diagnosis, which is still low, but comparable with data from a recent study among a large cohort of IBD patients which showed that only 25% was part of a surveillance program [25].

Although the increased risk of CRC in IBD patients is well established, studies on this topic are hampered by the fact that IBD-associated CRC is relatively rare. A prospective study should enrol large numbers of IBD patients at risk to produce enough IBD-associated CRC cases for meaningful risk factor analysis. The current retrospective study is unique because it reports the largest cohort of IBD-associated CRC in literature. This enabled us to describe and compare large subgroups of patients with IBD-associated CRC such as patients from referral centers and general hospitals. Nonetheless, this study design had limitations as well. We did not compare our data with a matched control cohort. Furthermore, data regarding disease extent and severity could only be obtained from endoscopy and histology charts, potentially introducing bias. This potential bias, however, is present in both cohorts, and will therefore probably not interfere with the outcomes. Due to the retrospective study design, data regarding family history of CRC and the presence of post-inflammatory polyps was missing in several cases.

In summary, we report that patients with IBD-associated CRC treated in RH's have a more severe course of IBD before CRC is diagnosed. Although both IBD and CRC were diagnosed at an earlier age in RH patients, the more severe course of IBD did not lead to a shorter interval between IBD and CRC diagnosis. Therefore, it can be questioned whether parameters of disease severity should be used for risk stratification in IBD patients, who are considered eligible for surveillance colonoscopies.

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Chapter 2

Chapter 3

Incidence of interval colorectal cancer among inflammatory bowel disease patients enrolled in a colonoscopic surveillance program

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Manuscript in preparation

ABSTRACT

Introduction

Colonoscopic surveillance is recommended for patients with longstanding inflammatory bowel disease (IBD) due to the increased risk of colorectal cancer (CRC). To study the effectiveness of this strategy, we determined the incidence of CRC after a negative surveillance colonoscopy (interval CRC).

Methods

Ulcerative colitis (UC) and Crohn's disease (CD) patients enrolled in a surveillance program were identified using the patients' medical records. Patients were followed from the first surveillance colonoscopy until the last surveillance, colectomy or CRC. Etiological factors potentially explaining the occurrence of CRC were categorized as inadequate procedures (i.e. inadequate bowel preparation), inadequate surveillance (CRC occurring outside the appropriate surveillance interval) or inadequate dysplasia management (CRC diagnosed in the same colonic segment as a previous diagnosis of dysplasia). The remaining cases of CRC were classified as interval CRCs.

Results

A total of 1,273 IBD patients (34% CD and 66% UC) were enrolled in a surveillance program and underwent 4327 surveillance colonoscopies comprising 6,823 years of follow-up. CRC was diagnosed in 17 patients (1.3%), with an incidence of 2.5 per 1000 follow-up years. One or more etiological factors potentially explaining the occurrence of CRC were identified in 12 patients (70%). This was inadequate colonoscopy in four patients (24%), inadequate surveillance interval in nine patients (53%) and inadequate dysplasia management in two patients (12%). The remaining five cases of CRC (30%) were classified as interval CRCs, with an overall incidence of 0.2 per 1000 years of follow-up.

Conclusion

The incidence of CRC among IBD patients enrolled in a surveillance program was low, which supports the implementation of longer surveillance intervals. However, the fact that one third of all CRC cases were interval cancers underscores the need for a variable surveillance interval based on the presence of risk factors for CRC.

INTRODUCTION

Both ulcerative colitis (UC) and Crohn's colitis (CD) are associated with an increased risk of developing colorectal cancer (CRC).[1, 2] Therefore, regular endoscopic surveillance is recommended aimed at achieving a reduction in CRC-related deaths by detecting and treating premalignant lesions (dysplasia) and early stage asymptomatic CRC.[3, 4]There is much debate on whether this strategy is (cost) effective, mainly because the evidence supporting the effectiveness of surveillance is limited. [5, 6]

The lack of solid evidence for CRC surveillance in IBD patients is also reflected by the differences in the recommended surveillance intervals of the recently updated British (BSG) and American (AGA) surveillance guidelines.[3, 4] The BSG guideline adopted a risk-stratified approach based on known risk factors for IBD-associated CRC with corresponding surveillance intervals of 1, 3 and 5 years, whereas the authors of the AGA guidelines state that optimal surveillance intervals cannot be clearly defined and therefore recommend that surveillance should be performed every 1 to 3 years. Determining the optimal surveillance interval is important because a shorter interval leads to unnecessary high costs and burden the patient, whereas a longer interval could lower the effectiveness of surveillance and may result in an increased incidence of CRC detected between two surveillance colonoscopies (interval CRC).

Since a direct prospective comparison between different surveillance intervals will probably never be performed, data on the occurrence of interval CRCs among IBD patients undergoing regular surveillance could provide vital information on whether current surveillance protocols are effective.[7] The occurrence of interval CRCs among IBD patients undergoing surveillance is largely unknown, although one study reported that as much as 50% of the CRCs diagnosed during the surveillance program were interval CRCs.[8] These data go back to the 1970's however, and it is not clear if these results can be extrapolated to the present clinical situation.

The aim of the current study is therefore to establish the incidence of interval CRCs in a cohort of IBD patients undergoing regular colonoscopic surveillance. Furthermore, we aimed to identify procedure-related factors potentially explaining the occurrence of CRC.

METHODS

Patients

All patients with a diagnosis of Crohn's disease (CD) or ulcerative colitis (UC) from 5 university hospitals and 2 general hospitals were identified using the Diagnosis Treatment Combinations (DTCs) for IBD. DTC's are based on the International Classification of Disease and can be consid-

ered the Dutch version of the Diagnosis Related Groups (DRGs) as used in other countries, e.g. the United States.

The medical records and endoscopy reports of all patients with a DTC code for CD or UC were reviewed to confirm the IBD diagnosis and to assess whether patients had a valid indication for surveillance colonoscopy according to the new AGA and BSG guidelines. Since the new BSG and AGA guidelines do not concur with regard to the beginning of surveillance (10 and 8 years after the onset of colitis symptoms, respectively), we considered patients with a disease-duration of at least 8 years eligible for surveillance. Patients with colitis and a concomitant diagnosis of primary sclerosing cholangitis (PSC) were considered to have an indication for surveillance immediately following diagnosis while patients with proctitis or proctosigmoiditis (UC patients) or with involvement of less than 30% of the colonic mucosa (CD patients) were considered to have no indication for surveillance and were excluded.

Furthermore, patients with any previous diagnosis of neoplasia (other than discrete solitary sessile or pedunculated polyps suggestive of sporadic adenomas and containing adenomatous tissue on histology) or (sub)total colectomy prior to the first surveillance colonoscopy were excluded.

Demographic and clinical data were collected from the medical records and included date of IBD diagnosis, type of IBD, disease extent prior to the start of surveillance, family history of CRC, medication use and a concomitant diagnosis of PSC. In UC and IBD-unclassified (IBDU) patients, disease extent was defined as either left-sided or extensive (inflammation distal or proximal to the splenic flexure, respectively). In patients with CD, involvement of 3 or more anatomical parts of the colon was considered extensive disease, whereas involvement of 1 or 2 sections was considered limited disease.

Surveillance colonoscopies

During the study period all participating centers performed surveillance in accordance with international guidelines, i.e. complete colonic inspection with or without the use of chromoendoscopy and biopsy sampling of all areas suspicious for neoplasia as well as sampling of 4 random biopsies every 10 cm when chromoendoscopy was not performed.

All endoscopy reports were reviewed to confirm that patients underwent at least two surveillance colonoscopies. A surveillance colonoscopy was defined as a procedure with the clear intention to detect neoplasia (explicitly stated as the indication for the colonoscopy and/or by the use of chromoendoscopy or taking four quadrant random biopsies every 10 cm).

Potential quality indicators such as cecal intubation and the number of random biopsies were collected from the endoscopy reports. Insufficient bowel preparation was recorded if this was stated as such in the endoscopy report. Furthermore, known endoscopic risk factors for

IBD-associated CRC were collected such as the extent and severity of inflammation and the presence of post inflammatory polyps or strictures.
For all lesions that were detected during follow-up and suspected of containing dysplasia, the location, endoscopic description and treatment were recorded. Based on the pathology report, lesions were categorized as non-dysplastic, indefinite for dysplasia (IFD), low-grade dysplasia (LGD), high-grade dysplasia (HGD) or colorectal cancer (CRC). Endoscopically visible lesions containing LGD were subdivided in adenoma-like mass (discrete solitary sessile or pedunculated polyps resembling sporadic adenomas and containing adenomatous tissue on histology) and non-adenoma-like mass (all other endoscopic descriptions, i.e. plaque-like lesions, irregular masses).

CRC characteristics

Patients were followed-up from the date of the first surveillance colonoscopy until the last surveillance colonoscopy, (sub)total colectomy or the diagnosis of CRC. Each diagnosis of CRC within the surveillance program was categorized according to its most probable explanation, adopted from similar studies on sporadic CRC.[9] Procedure-related CRCs were defined as inadequate bowel preparation, incomplete intubation or less than 10 random biopsies sampled during the surveillance colonoscopy prior to the CRC diagnosis. Inadequate surveillance was defined as CRC occurring outside the appropriate surveillance interval. Since the 2002 BSG guidelines were followed during the study period, we considered a one-year surveillance interval appropriate for patients with prior dysplasia or a concomitant diagnosis of PSC and a two-year surveillance interval for the remaining patients. We also stratified patients in the 1-year, 3-year and 5-year surveillance interval based on the risk factors described in the new BSG guidelines. Inadequate dysplasia management was defined as CRC diagnosed in the same colonic segment as a previous diagnosis of dysplasia.

Definition of interval CRC

All cases of CRC detected within the appropriate surveillance interval, after an adequately performed surveillance colonoscopy were considered interval cancers. In other words, these were cases of CRC that could only be explained by either missed lesions during the preceding colonoscopy or rapid progression to cancer within the surveillance interval. In a second analysis we defined interval CRCs as advanced stage CRC (Dukes stage C or D) diagnosed within the appropriate surveillance interval, in accordance with the definition used by Rutter.[8]

RESULTS

Surveillance cohort

A total of 1273 IBD patients were identified that were enrolled in a surveillance program, of which 434 patients had CD (34%), 804 had UC (63%) and 35 had IBD-U (3%). Baseline characteristics are shown in table 1.

During a mean follow-up time of 5.3 years (±3.0 years), 4327 surveillance colonoscopies were performed in the study population comprising 6823 years of follow-up. Surveillance was performed with random biopsy sampling in 3887 procedures (90%) during which a mean of 27 biopsies (±12) were sampled (table 2). The remaining 440 surveillance procedures (10%) were performed using chromoendoscopy with targeted biopsies. The cecal intubation rate was 99% and bowel preparation was deemed adequate in 87% of procedures.

Table 1: baseline characteristics of patients undergoing surveillance

	N (%)
Number of patients	1273
Male gender	676 ()
IBD diagnosis	
Ulcerative colitis	804 (63)
Distal splenic flexure	269 (33)
Proximal splenic flexure	456 (57)
Unknown	79 (10)
Crohn's colitis	434 (34)
Segmental colitis <50%	159 (37)
Segmental colitis >50%	228 (52)
Unknown	47 (11)
Indeterminate colitis	35 (3)
Segmental colitis <50%	16 (46)
Segmental colitis >50%	18 (51)
Unknown	1 (3)
Age at first surveillance colonoscopy, yr (mean ± SD)	45.8 (±12.9)
Duration of IBD at first surveillance colonoscopy , yr (mean ± SD)	17.4 (±3.9)
Concomitant diagnosis of PSC	173 (±14)
Duration of follow-up, yr (mean ± SD)	5.3 (±3.0)

IBD, inflammatory bowel disease; SD, standard deviation; PSC, primary sclerosing cholangitis.

Table 2: Characteristics of the surveillance colonoscopies

	N (%)
Total number of surveillance colonoscopies	4327 (100)
WLE + random biopsies	3887 (90)
Chromoendoscopy	440 (10)
Number of surveillance colonoscopies (median, range)	2 (1 – 11)
Cecal intubation	4288 (99)
Suboptimal bowel preparation	566 (13)
Number of biopsies per colonoscopy (mean ± SD)	27 (± 12)
Endoscopic inflammation	
No active inflammation	2847 (66)
Mild	1182 (27)
Moderate to severe	298 (7)
Histologic inflammation	
No active inflammation	2383 (55)
Mild	1429 (33)
Moderate or severe	276 (6)
unknown	239 (6)
Post inflammatory polyps	926 (21)
Neoplasia	438 (10)
IFD	49 (1)
LGD	354 (8)
HGD	18 (0.4)
CRC	14* (0.3)

SD, standard deviation.

* three cases of CRC were detected as an incidental finding during colonoscopy to assess disease activity or (sub) total colectomy for therapy refractory disease activity

Incidence of dysplasia during follow-up

LGD was detected during 354 surveillance colonoscopies in 265 patients (21%) during follow-up, comprising 482 lesions with LGD. These lesions were classified as 339 adenomas, 43 non-adenoma-like lesions and 100 episodes of flat dysplasia originating from a total of 107.969 random biopsies. The incidence of LGD was 52 per 1000 years of follow-up.

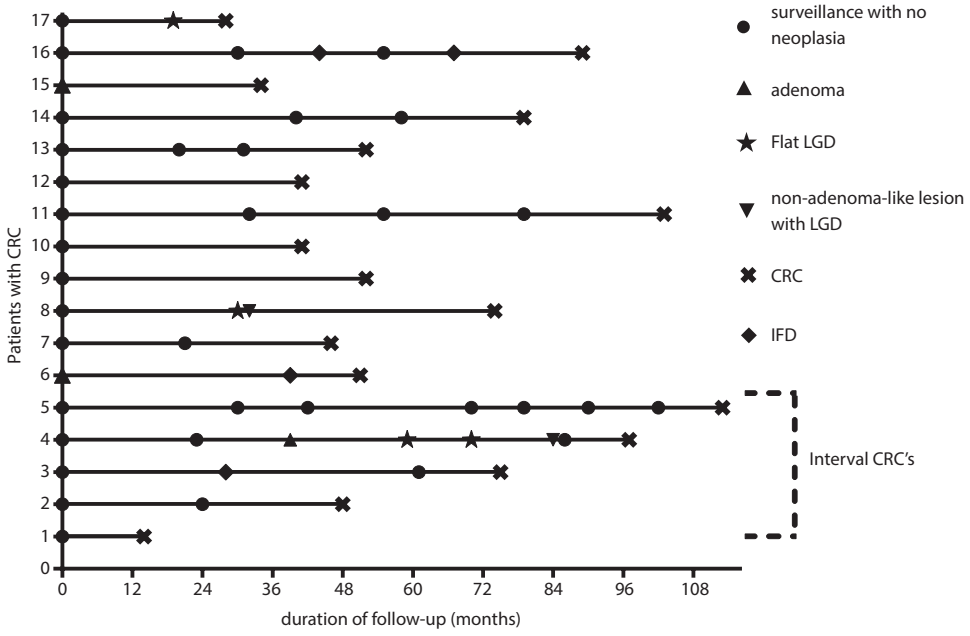
High-grade dysplasia was detected during 19 surveillance colonoscopies in 19 patients (1.5%), with an incidence of 2.8 per 1000 years of follow-up. HGD was found in random biopsies in 2 patients (0.2%) and in a lesion in the remaining 17 patients (1.3%).

Incidence of CRC during follow-up

In total, 18 CRCs were diagnosed in 17 patients (1.3%) at a median age of 55 years with an incidence of 2.5/1000 years of follow-up (table 3). CRC was detected during a scheduled surveillance colonoscopy in 14 patients. The remaining three CRCs were detected during colonoscopy for an indication other than surveillance, as an incidental finding during ileocecal resection or after analysis of liver metastases with an unknown primary tumor.

A schematic presentation of all surveillance colonoscopies and diagnoses of dysplasia prior to the CRC diagnosis for each individual patient are shown in figure 1. The median interval between the last surveillance colonoscopy and the CRC diagnosis was 22 months (range 9 – 42 months).

Figure 1: All surveillance colonoscopies and episodes of dysplasia prior to the diagnosis of CRC



CRC, colorectal cancer; LGD, low-grade dysplasia; IFD, indefinite for dysplasia

Table 3: CRC characteristics

	N (%)
Patients diagnosed with CRC	17 (100)
Male Gender	12 (71)
Mean age at diagnosis (years, mean +-SD)	55 (12.6)
IBD diagnosis	
Ulcerative colitis	8 (47)
Crohn's colitis	8 (47)
Indeterminate colitis	1 (6)
Interval between last surveillance and CRC diagnosis (months, median, range)	21 (5 – 42)
Tumor location*	
Cecum	5 (28)
Ascending colon	6 (33)
Transverse colon	1 (6)
Descending colon	1 (6)
Rectosigmoid	4 (21)
Unknown	1 (6)
Tumor stage	
Dukes A	3 (18)
Dukes B	5 (29)
Dukes C	4 (24)
Dukes D	4 (24)
Unknown	1 (6)

* One patient was diagnosed with two CRCs wich were counted seperately

Etiology of CRC

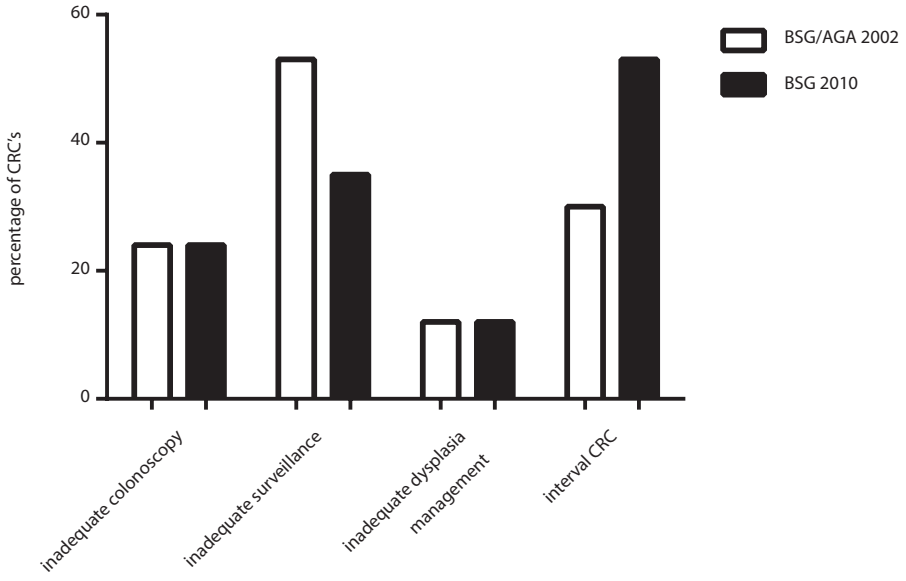
Inadequate colonoscopy was identified as the most likely cause in four patients (24%, figure 2). Inadequate surveillance interval was identified as an etiological factor in nine patients (53%) according to the previous BSG and AGA guidelines and in six patients (35%) when patients were stratified according to the surveillance intervals of the updated BSG guideline.

Two patients (12%) had either flat LGD originating from random biopsies (n=1) or LGD in an endoscopically-resected lesion (n=1) during the surveillance procedure directly prior to the CRC diagnosis, which was located in the same colonic segment as the CRC (inadequate dysplasia management).

Five cases of CRC (30%) could be classified as interval CRC according to our definition of CRC

diagnosed within the appropriate surveillance interval after an adequately performed surveillance colonoscopy, resulting in an incidence of 0.2 interval CRCs per 1000 years of follow-up. Interestingly, all five cases were advanced stage CRC, Dukes C or D, and could therefore be classified as interval cancers according to our second definition for interval CRC as well. Since the remaining three cases of CRC diagnosed at an advanced stage were all diagnosed outside the appropriate surveillance interval, the rate of interval CRC was equal for both definitions. Three cases of interval CRC were identified during a regular surveillance colonoscopy at the appropriate interval, one during a colonoscopy for abdominal pain and one for liver metastases with an unknown primary tumor. Three interval CRCs (60%) were located in the right colon, as compared to eight of the remaining 12 CRCs (67%). Three patients with interval CRC had UC (60%), none had a concomitant diagnosis of PSC and two had post-inflammatory polyps (40%).

Figure 2: CRC cases subdivided based on the most likely etiological factor.



DISCUSSION

This study shows that the incidence of CRC among IBD patients enrolled in a surveillance program is low, with only 17 cancers detected during 6823 years of follow-up.

The majority of CRC cases (70%) could be explained by an inadequate surveillance procedure prior to the CRC diagnosis (i.e. inadequate bowel prep), inadequate surveillance interval or inadequate dysplasia management. This suggests that the incidence of CRC could have been even lower if surveillance was performed strictly according to the guidelines in all patients. Several studies have highlighted the problem of adherence to the CRC surveillance guidelines in patients with longstanding IBD.[10-13] The relative large number of potentially preventable CRCs in the present surveillance cohort stresses the importance of increasing the awareness of both patients and gastroenterologists for adequately performed surveillance at the appropriate interval.

The CRC incidence of 2.5 per 1000 person years of follow-up in the current study is substantially lower than the incidence of 5.9 per 1000 person-years of follow-up reported by Rutter et al., the largest surveillance cohort to date, despite a similar study design.[8] This can probably be explained by the fact that our study included data from the year 2000 onwards, whereas the Rutter cohort dates back to the 1970 's. Large population-based cohorts show that the incidence of CRC among IBD patients is gradually decreasing over time, most likely reflecting the improvement in the quality of endoscopes and colonoscopic performance combined with improvements in anti-inflammatory medication which might well explain the lower incidence of CRC in our study.[14, 15]

Only five cases of interval CRC, defined as advanced stage CRC despite adequate surveillance at the appropriate interval prior to the CRC diagnosis, were identified. Although these CRCs constituted 30% of the total number of CRCs in our cohort, the incidence of 0.7 per 1000 follow-up years is considerably lower than the 2.7 per 1000 follow-up years reported by Rutter.[8] Again, the fact that our data originate from a more recent era with improved endoscopes and most likely a lower overall risk of CRC seems a logical explanation for this discrepancy. Another more recently published study on interval CRCs among IBD patients identified all cases of CRC diagnosed during or within 36 months after colonoscopy and compared the percentage of CRCs diagnosed 6-36 months after colonoscopy (interval CRCs) out of the total number of CRCs between patients with and without IBD.[16] In this study, the percentage of interval CRCs was three times higher in IBD patients compared to those without IBD, stressing the importance of close surveillance in these patients. This study lacked data on the colonoscopies prior to the CRC diagnosis including the number of biopsies, bowel prep and the data to assess the appropriate surveillance interval for each patient. Therefore, the number of interval CRCs cannot be

compared to our study.

Reports on the incidence of interval CRC (defined as CRC following a negative colonoscopy) among patients without IBD range between 0.2 and 1.2 per 1000 years of follow-up.[17, 18] The incidence of true interval CRCs in our study of 0.7/1000 years of follow-up is therefore comparable to the interval CRC incidence in patients undergoing surveillance for sporadic CRC.

Two likely explanations for the occurrence of interval CRCs are either missed dysplastic lesions or cancer developing in the time between two surveillance colonoscopies. Although it has been suggested that the carcinogenesis in IBD-associated CRC might be accelerated as compared to sporadic CRC, the fact that dysplasia in IBD patients is often flat and can therefore be overlooked more easily offers a plausible explanation for the occurrence of interval CRCs in these patients.[19, 20] The low rate of interval CRC in our cohort, suggests that missed lesions do not pose a large problem when surveillance is performed at an interval of 1-2 years. Although optimal surveillance intervals cannot be deduced from our study, the low incidence of both CRC and interval CRC might justify surveillance intervals of three or even five years in patients without additional risk factors, as suggested in the updated BSG and ECCO guidelines.[3, 21]

Our study has several limitations that need to be addressed. The low overall incidence of CRC in our surveillance cohort precluded a meaningful analysis of risk factors for interval CRCs.

The retrospective nature of this study resulted in several limitations as well. First, the criteria for inadequate surveillance were based on endoscopy reports rather than a standardized protocol, potentially introducing reporter bias regarding adequate bowel preparation and cecal intubation rate. Another potential confounding factor might be the classification of lesions as either adenoma-like or non-adenoma-like as this was based on the description of the lesion taken from the endoscopy report.

Furthermore, we were unable to identify the reasons why in some cases the recommended surveillance interval was not used. This information is important because this could guide an intervention towards either better education of patients regarding the need for surveillance or better understanding of the guidelines among gastroenterologists.

In conclusion, this study shows that the incidence of CRC is low among IBD patients undergoing regular endoscopic surveillance. A longer surveillance interval of up to 5 years as recommended in the current BSG and ECCO guidelines for surveillance seems therefore appropriate. However, the fact that one third of all CRC cases appear to be interval carcinomas underscores the need to further identify risk factors associated with development of interval cancer.

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Chapter 4

Neoplasia yield and colonoscopic workload of surveillance regimes for colorectal cancer in colitis patients: a retrospective study comparing the performance of the updated AGA and BSG guidelines

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Published in Inflammatory Bowel Diseases

ABSTRACT

Background

Due to the increased risk of colorectal cancer (CRC) colonoscopic surveillance is recommended for patients with ulcerative and Crohn's colitis. Since surveillance intervals differ considerably between the recently updated American Gastroenterological Association (AGA) and British Society of Gastroenterology (BSG) guidelines, we compared the neoplasia yield and colonoscopic workload of these guidelines.

Methods

IBD patients undergoing surveillance were identified using medical records. Patients were stratified according to the BSG and AGA guidelines and corresponding colonoscopic workload was calculated based on risk factors present during follow-up. The incidence of colitis-associated neoplasia (CAN), defined as low-grade dysplasia in flat mucosa or a non-adenoma-like mass, high-grade dysplasia or CRC was compared between the risk groups of either guideline.

Results

In total, 1018 IBD patients that underwent surveillance were identified. Employing the AGA surveillance intervals, 64 patients (6%) were assigned to annual and 954 patients (94%) to biannual surveillance, resulting in 541 colonoscopies/year. The yield of CAN was 5.3% and 20.3% in the low- and high-risk group respectively (p=0.02).

Using the BSG surveillance intervals, 204 patients would receive surveillance annually (20%), 393 patients every three years (39%) and 421 patients every five years (41%), resulting in 420 colonoscopies/year which is 22% lower than the AGA guidelines. The yield of CAN was 3.6%, 6.9% and 10.8%, for the low-, intermediate- and high-risk group group respectively (p=0.26).

Conclusion

Although the BSG surveillance intervals offer the advantage of a lower colonoscopic workload, the risk stratification of the AGA seems superior in distinguishing patients at higher risk of CAN.

INTRODUCTION

Patients with longstanding extensive ulcerative colitis and Crohn's colitis are at an increased risk of developing colorectal cancer (CRC).[1] For this reason, colonoscopic surveillance has been advocated, although solid evidence that this indeed prolongs survival is lacking.[2] In 2002, both the American Gastroenterological Association (AGA) and the British Society of Gastroenterology (BSG) published their first guidelines describing which patients should undergo surveillance and how this should be performed.[3,4] Although there were some differences regarding the recommended surveillance intervals between these guidelines, both stated that regular surveillance with an interval between 1 and 3 years should be performed after 8-10 years of disease duration in case of at least extensive colitis and after 15-20 years in patients with left-sided disease. Since CRC risk in ulcerative colitis and Crohn's colitis seems to be similar for comparable extent and duration, these guidelines are considered to be applicable to patients with Crohn's colitis as well.[5]

Both the British (BSG) and the American (AGA) guidelines were recently updated to implement new endoscopic techniques and improved risk stratification.[6,7] Although both guidelines still recommend regular surveillance in all patients with extensive colitis and now endorse the use of chromoendoscopy, there is no consensus regarding the intervals between surveillance colonoscopies. The new BSG guidelines adopt an algorithm based on established risk factors for colitis-associated CRC to stratify patients in a high-, intermediate- and low-risk group with adjusted surveillance intervals. The most striking difference as compared to the previous BSG guidelines is that the low risk group is now recommended to undergo surveillance every 5 years. The same risk factors are mentioned in the updated AGA guidelines, but the authors state that optimal surveillance intervals cannot be clearly defined and therefore recommend surveillance every 1-3 years.

Although longer surveillance intervals offer an advantage in terms of colonoscopic workload and cost reduction, the drawback could be a higher number of interval cancers, thereby reducing its effectiveness. Since a head-to-head comparison of the updated BSG and AGA guidelines will probably never be performed, we aimed to establish which guidelines can identify patients at risk for neoplasia best. Furthermore, we assessed differences in colonoscopic workload between the new AGA and BSG guidelines.

METHODS

Patients

All patients with a diagnosis of Crohn's disease (CD) or ulcerative colitis (UC) from 3 university hospitals and 2 general hospitals were identified using the Diagnosis Treatment Combinations (DTCs) for IBD. DTC's are based on the International Classification of Disease, 9th Revision and can be considered the Dutch version of the Diagnosis Related Groups (DRGs) which are used in other countries, e.g. 1he United States. [8]

The medical records and endoscopy reports of all patients with a DTC code for CD or UC were reviewed to confirm the IBD diagnosis and to assess whether patients had a valid indication for surveillance colonoscopy according to the new AGA and BSG guidelines. Since the new BSG and AGA guidelines do not concur with regard to the beginning of surveillance (10 and 8 years after the onset of colitis symptoms, respectively), we considered patients with a disease duration of at least 8 years eligible for surveillance. Patients with colitis and a concomitant diagnosis of primary sclerosing cholangitis (PSC) were considered to have an indication for surveillance immediately following diagnosis while patients with proctitis or proctosigmoiditis (UC patients) or with involvement of less than 30% of the colonic mucosa in case of CD were considered to have no indication for surveillance and were excluded.

During the study period all participating centers performed surveillance in accordance with international guidelines, i.e. complete colonic inspection including biopsy sampling of all areas suspicious for neoplasia with or without the use of chromoendoscopy as well as sampling of 4 random biopsies every 10 cm when chromoendoscopy was not performed. Standard bowel preparation consisted of 4 litres of polyethylene glycol solution.

All endoscopy reports were reviewed to confirm that patients underwent at least one surveillance colonoscopy. A surveillance colonoscopy was defined as a procedure with the clear intention to detect neoplasia (explicitly stated as the indication for the colonoscopy and/or by taking four quadrant random biopsies every 10 cm). Exclusion criteria were any previous diagnosis of neoplasia (other than discrete solitary sessile or pedunculated polyps resembling sporadic adenomas and containing adenomatous tissue on histology) or (sub)total colectomy prior to the first surveillance colonoscopy.

Data collection

Demographic and clinical data were collected from the medical records and included date of IBD diagnosis, type of IBD, disease extent prior to the start of surveillance, family history of CRC, medication use and a concomitant diagnosis of PSC. Disease extent prior to the first surveillance colonoscopy was defined as the maximum extent according to either the histology

or endoscopy reports. In UC and IBD-unclassified (IBDU) disease extent was defined as either left-sided or extensive (inflammation distal or proximal to the splenic flexure, respectively). In patients with Crohn's colitis, involvement of 3 or more anatomical parts of the colon was considered extensive disease, whereas involvement of 1 or 2 sections was considered limited disease.

Neoplasia

For all suspected dysplastic lesions the location and endoscopic description was recorded. Based on the pathology report, lesions were categorized as non-dysplastic, indefinite for dysplasia (IFD), low-grade dysplasia (LGD), high-grade dysplasia (HGD) or colorectal cancer (CRC). Endoscopically visible lesions containing LGD were subdivided in adenoma-like mass (discrete solitary sessile or pedunculated polyps resembling sporadic adenomas and containing adenomatous tissue on histology) and non-adenoma-like mass (all other endoscopic descriptions, i.e. plaque-like lesions, irregular masses). Our primary endpoint was colitis-associated neoplasia (CAN), defined as patients developing a non-adenoma-like mass containing LGD, flat dysplasia (LGD or HGD), HGD or CRC.

Follow-up

Patients were followed from the date of the first surveillance colonoscopy until one of the following endpoints: 1. last surveillance colonoscopy 2. (sub)total colectomy 3. death 4. diagnosis of CAN.

Endoscopic risk factors were scored at each surveillance colonoscopy during follow-up including extent and severity of inflammation, presence of post-inflammatory polyps and strictures. Severity of endoscopic and histologic inflammation was scored as no inflammation, mild, moderate or severe inflammation as specified in the endoscopy and histology report. Patients were scored as positive for each risk factor when this was present at one or more surveillance colonoscopies during follow-up.

Surveillance intervals

The enrolled patients were stratified in the risk groups as specified in the new AGA and BSG guidelines to calculate the colonoscopic workload of surveillance and to compare the yield of CAN between the risk groups. Details about the risk factors employed by either guideline are provided in figure 3.

Statistical analysis

Baseline patient and endoscopic characteristics during the surveillance colonoscopies were

analyzed using standard descriptive statistics. The cumulative incidence of CAN was calculated for the different risk-strata according to the BSG and AGA guidelines using Kaplan Meier analysis and comparisons between risk groups were made with log rank testing. Patients who did not develop CAN were censored at the moment of last surveillance colonoscopy or colectomy. To test the discriminative power of the risk groups of either guideline in identifying patients with CAN the c-statistic was calculated using Cox- regression analysis.
To calculate the colonoscopic workload of the BSG and AGA guidelines, the contribution of each patient to the average annual number of surveillance colonoscopies was calculated based on the intervals described in either guideline. Univariate and multivariate analysis was performed using Cox regression analysis to identify predictors for the development of CAN. A two-sided p-value <0.05 was considered statistically significant. Data were analysed using SPSS version 20 for Windows.

Ethical considerations

This study was carried out with the approval of and in accordance with the ethical guidelines of the medical ethical committee of our institution.

RESULTS

Patients

Our search identified 4514 patients with a DTC code for Crohn's disease or ulcerative colitis. Surveillance was performed in 1018 patients (23%), of whom 408 had Crohn's colitis (40%), 573 had ulcerative colitis (56%) and 37 had indeterminate colitis (4%). Baseline characteristics of the study population are shown in table 1.

Table 1: baseline characteristics of patients undergoing surveillance

	N (%)
Number of patients	1018 (100)
Male gender	491 (48)
Type of hospital	
Referral center	737 (73)
General hospital	281 (27)
IBD diagnosis	
Ulcerative colitis	573 (56)
Distal splenic flexure	252 (44)
Proximal splenic flexure	309 (54)
Unknown	12 (2)
Crohn's colitis	408 (40)
Segmental colitis <50%	173 (42)
Segmental colitis >50%	216 (53)
Unknown	19 (5)
Indeterminate colitis	37 (4)
Segmental colitis <50%	17 (46)
Segmental colitis >50%	19 (51)
Unknown	1 (3)
Age at first surveillance colonoscopy, yr (mean ± SD)	46.7 (±12.6)
Duration of IBD at first surveillance colonoscopy , yr (mean ± SD)	16.7 (±8.3)
Concomitant diagnosis of PSC	64 (6)
Medication use (>3 months)	
5-ASA	880 (86)
AZA	480 (47)
Methotrexate	75 (7)
Biologicals	165 (16)
Duration of follow-up, yr (median, range)	2.6 (0 – 12.5)
Partial or total colonic resection during follow-up	78 (8)

IBD, inflammatory bowel disease; SD, standard deviation; PSC, primary sclerosing cholangitis.

Surveillance colonoscopies

In total, 2371 surveillance colonoscopies were performed during follow up, with a median of 2 surveillance colonoscopies per patient (range 1-10). Bowel preparation was judged to be adequate in 90% of surveillance colonoscopies and the cecal intubation rate was 97%. Chromoendoscopy was used in 53 surveillance colonoscopies (2%) while random biopsies were taken in the remaining 2318 colonoscopies (98%).

Active endoscopic inflammation was present in 777 (33%) of surveillance colonoscopies and active histologic inflammation in 947 (40%) surveillance colonoscopies (Table 2). Post-inflammatory polyps were encountered in 506 surveillance colonoscopies in 257 patients (25%).

Table 2: Endoscopic characteristics

	N (%)
Number of colonoscopies	2371 (100)
Cecal intubation	2301 (97)
Suboptimal bowel preparation	244 (10)
Number of biopsies per colonoscopy (mean ± SD)	
Random biopsy protocol	27 (±10)
Chromoendoscopy protocol	9 (±5)
Endoscopic inflammation	
No signs of previous or present inflammation	266 (11)
Quiescent disease	1328 (56)
Mild	621 (26)
Moderate to severe	156 (7)
Histologic inflammation	
No signs of previous or present inflammation	110 (5)
Quiescent disease	1314 (55)
Mild	790 (33)
Moderate or severe	157 (7)
Post inflammatory polyps	506 (21)
Stricture	
Ulcerative colitis	10 (1)
Crohn's colitis	65 (3)

SD, standard deviation.

Neoplasia during follow-up

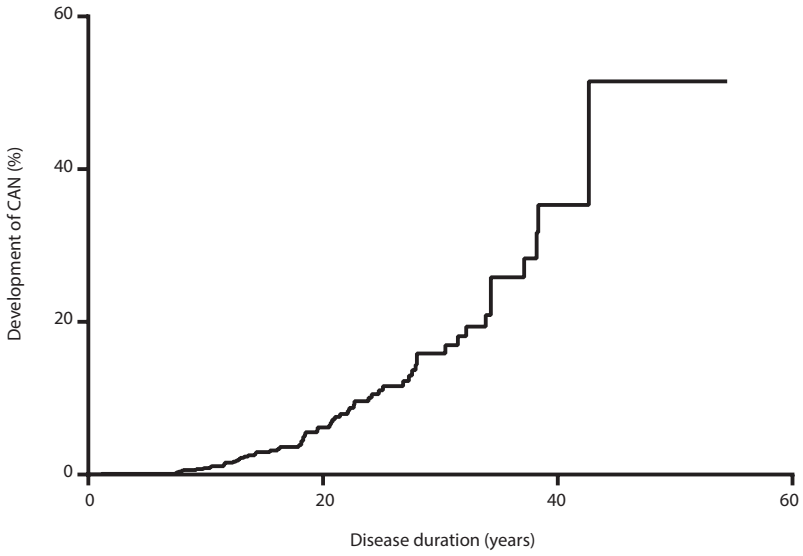
Neoplasia was detected during one or more of the follow-up surveillance colonoscopies in 173 patients (15%) (Table 3). Based on the endoscopy and histopathology reports, 64 (5%) patients developed CAN during follow-up: 11 patients developed CRC, 6 patients HGD, 32 patients flat LGD and 15 patients a non-adenoma-like mass with LGD (table 3). The remaining 109 patients developed either IFD (11 patients) or an adenoma-like mass containing LGD (98 patients). The cumulative incidence of CAN by disease duration was 0.9% at 10 years, 6.2% at 20 years, 16.9% at 30 years and 35.3% at 40 years (figure 1).

Table 3: Neoplasia during follow-up, based on the maximal grade of dysplasia

	Random biopsy protocol (n=965)	**Chromoendoscopy protocol (n=53)**
Patients diagnosed with neoplasia	162 (17%)	11 (21%)
Indefenite for dysplasia	11 (1%)	0 (0%)
Low-grade dysplasia		
Adenoma-like mass	89 (9%)	9 (17%)
Flat dysplasia	31 (3%)	2 (4%)
Non-adenoma-like mass	15 (1%)	0 (0%)
High-grade dysplasia	6 (0.6%)	0 (0%)
CRC	11 (1%)	0 (0%)
Dukes A	1	-
Dukes B	5	-
Dukes C	3	-
Dukes D	2	-

CRC, colorectal cancer

Figure 1: Kaplan-Meier curve showing the development of colitis-associated neoplasia (CAN) by disease duration. Vertical lines represent events of CAN (colitis-associated neoplasia) defined as non-adenoma-like LGD, HGD or CRC.



Low-grade dysplasia

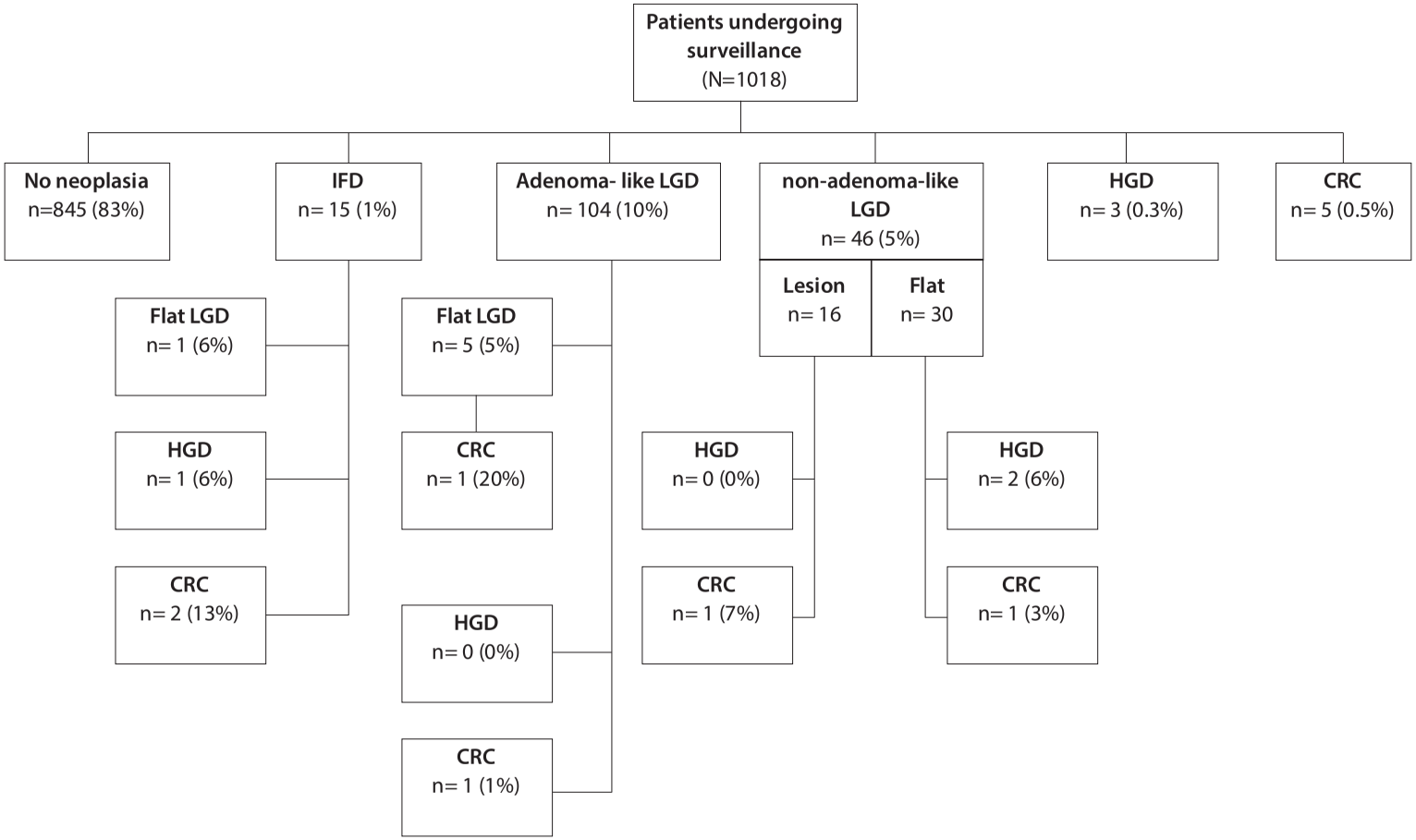
Flat LGD was diagnosed in 36 patients, of whom two patients progressed to HGD and two to CRC (figure 2). Flat LGD was unifocal in 26 patients (72%) and located distally to the splenic flexure in 19 patients (53%).

A non-adenoma-like mass containing LGD was found in 16 patients (nine proximal to the splenic flexure, median size 16 mm (range 2 - 30)) which was treated by colectomy in six patients. In none of these cases HGD or CRC was detected in the colectomy specimen. In 10 patients the lesion was treated endoscopically. In one of these patients, who was operated on for refractory disease three years after removal of the non-adenoma-like mass, CRC was unexpectedly diagnosed in the ileocoecal resection specimen.

High-grade dysplasia

HGD was diagnosed in six patients during follow-up (Figure 2). Two patients developed HGD in an adenoma-like mass, which was treated endoscopically in one patient and by colectomy in the other patient.

Figure 2: Flow chart showing the development of neoplasia during follow-up. LGD, low-grade dysplasia; HGD, high-grade dysplasia; CRC, colorectal cancer.



One patient developed flat HGD one year after diagnosis of flat LGD, which was not treated by colectomy due to advanced age. Two patients with HGD in a non-adenoma-like mass were found to have synchronous multifocal flat LGD in other segments of the colon. Both were treated by colectomy, which confirmed the diagnoses of LGD and HGD but revealed no additional advanced neoplasia.
In one patient HGD was diagnosed in biopsies surrounding a non-adenoma-like mass containing LGD. The colectomy specimen confirmed the presence of HGD and LGD with no additional diagnosis of CRC.

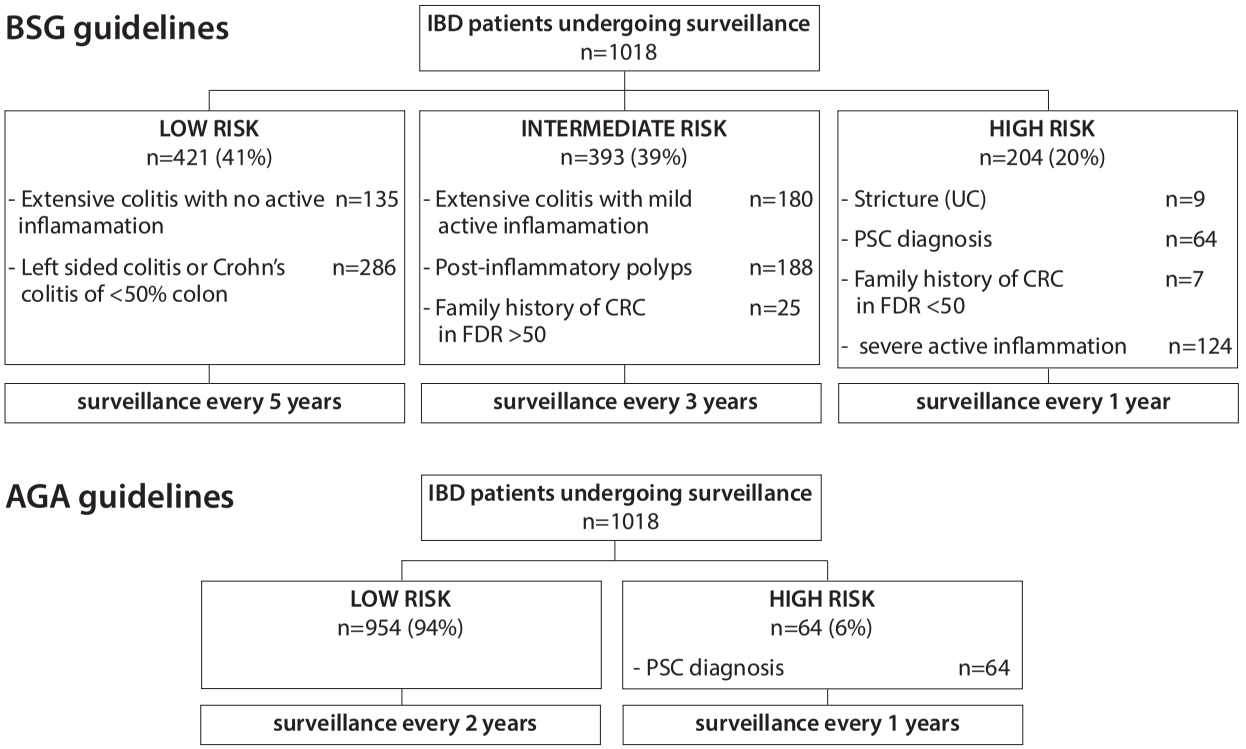
Colorectal cancer

A total of 12 CRCs were diagnosed in 11 patients during follow-up at a median age of 59 years (range 39 - 69) (figure 2). In five patients CRC was diagnosed without a prior diagnosis of neoplasia despite the fact that all patients underwent at least one surveillance colonoscopy before the CRC diagnosis with a median interval between the last surveillance colonoscopy and the CRC diagnosis of 26 months (range 20 – 47 months).
In six patients, dysplasia was detected during surveillance colonoscopies, prior to the diagnosis of CRC. The dysplasia diagnosis was flat IFD in two patients and LGD in four patients (figure 2).

Surveillance intervals according to the new BSG guidelines

Based on risk factors present during follow-up, surveillance intervals were determined according to the AGA and BSG guidelines in all 1018 patients. When applying the new BSG guidelines, 421 patients (41%) were assigned to the low-risk group (surveillance interval of 5 years), 393 patients (39%) to the intermediate-risk group (surveillance interval of 3 years) and 204 patients (20%) to the high-risk group (annual surveillance interval)(figure 3). CAN was detected in 15 low-risk patients (3.6%) (14 LGD, 0 HGD and 1 CRC), in 27 intermediate-risk patients (6.9%) (21 LGD, 0 HGD and 6 CRC) and in 22 high-risk patients (10.8%) (12 LGD, 6 HGD and 4 CRC).
The 5-year cumulative incidence of CAN was 5.6%, 7.2% and 9.9% for the low, intermediate and high-risk groups, respectively (figure 4) (low vs. high risk p=0.07, low vs. medium p=0.48 medium vs. high p=0.33, Log rank test). If the primary endpoint was limited to cases of HGD or CRC, the 5-year cumulative incidence was 0.7%, 1.8% and 4.3% for the low, intermediate and high-risk groups, respectively (figure 5) (low vs. high risk p<0.01, low vs. medium p=0.21 medium vs. high p=0.09, Log rank test). Employing the new BSG intervals resulted in an average annual workload of 420 surveillance colonoscopies, or 0.41 colonoscopies per patient per year. When the three risk groups were entered in a Cox regression analysis, the corresponding c-statistic was 0.55.

Figure 3: Flow chart showing the stratification of patients undergoing surveillance according to the AGA and BSG guidelines.



IBD, inflammatory bowel disease; CRC, colorectal cancer; FDR, first degree relative; UC, ulcerative colitis; PSC, primary sclerosing cholangitis.

Figure 4: Kaplan-Meier curve comparing the cumulative incidence of colitis-associated neoplasia (CAN) between the risk groups of the BSG guideline (left) and the AGA guideline (right). CAN is defined as non-adenoma-like LGD, HGD or CRC. Low-risk vs. high-risk p=0.07; Low vs. intermediate p=0.48; intermediate vs. high p=0.33 (BSG guideline). Low risk vs. high risk p=0.02 (AGA guideline)

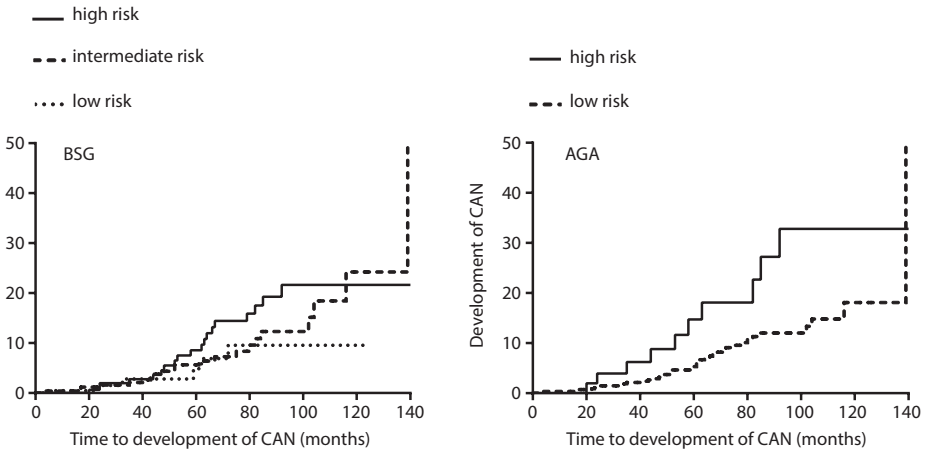
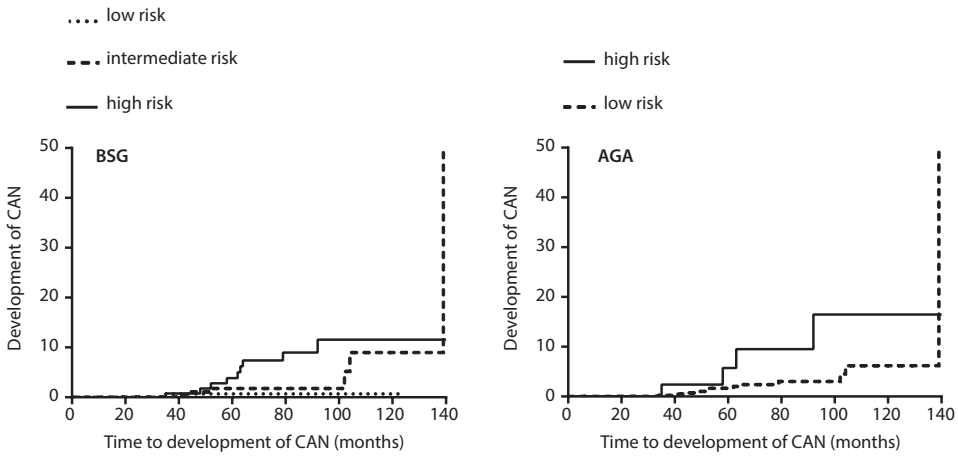


Figure 5: Kaplan-Meier curve comparing the cumulative incidence of high-grade dysplasia (HGD) or colorectal cancer (CRC) between the risk groups of the BSG guideline (left) and the AGA guideline (right). Low-risk vs. high-risk p<0.01; Low vs. intermediate p=0.21; intermediate vs. high p=0.09 (BSG guideline). Low risk vs. high risk p=0.02 (AGA guideline).



Surveillance intervals according to the new AGA guidelines

In accordance with the new AGA guidelines, 954 patients (94%) were assigned to the low-risk group and 64 patients (6%) to the high-risk group (figure 3). CAN was detected in 51 low-risk patients (5.3%) (39 LGD, 2 HGD, 10 CRC) and in13 high-risk patients (20.3%) (8 LGD, 4 HGD, 1 CRC). The 5-year cumulative incidence of CAN was 6.3% in the low-risk group and 18.7% in the high-risk group (p=0.02, log rank) (figure 4). If the primary endpoint was limited to cases of HGD or CRC, the 5-year cumulative incidence was 1.7% in the low-risk group and 7.2% in the high-risk group (p=0.02, log rank) (figure 5).

The average annual number of surveillance colonoscopies when applying the AGA intervals was 541, or 0.53 per patient per year when a surveillance interval once every two years was used for the low-risk group. When a surveillance interval of three years was used for the low-risk group the average annual number of surveillance colonoscopies drops to 382 or 0.38 per patient per year. Therefore, the colonoscopic workload when adopting the BSG guidelines is 22% less than the workload associated with the AGA guidelines if a surveillance interval once every two years for the low-risk group is used. When the two risk groups were entered in a cox proportional hazards model, the corresponding c-statistic was 0.57.

Factors associated with the development of CAN

Univariate analysis showed that the development of CAN was significantly associated with male gender (OR 1.7), a positive family history of CRC (OR 3.2), PSC (OR 2.6), the presence of strictures in UC patients (OR 4.5) and the absence of histological inflammation (OR 0.6). After multivariate analysis PSC, a family history of CRC, the absence of histological inflammation and presence of strictures in UC patients remained significantly associated with the development of CAN (table 4).

Table 4: Cox proportional hazard analysis of the association between several known risk factors and the incidence of colitis associated neoplasia.

	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Male gender	1.7 (1.0-2.9)	1.4 (0.80-2.5)
Ulcerative colitis (vs. Crohn's disease)	1.3 (0.8-2.2)	1.1 (0.6-2.1)
Extensive colitis	1.0 (0.6-1.8)	0.9 (0.5-1.9)
Endoscopic inflammation		
No inflammation	0.8 (0.5-1.3)	0.8 (0.5-1.5)
Mild	0.7 (0.4-1.1)	0.6 (0.3-1.1)
Moderate/severe	1.2 (0.6-2.3)	0.9 (0.4-2.1)
Histological inflammation		
No inflammation	0.6 (0.3-1.0)	**0.4 (0.2-0.8)**
Mild	0.8 (0.5-1.3)	0.6 (0.3-1.1)
Moderate/severe	1.2 (0.6-2.1)	1.1 (0.5-2.4)
Stricture (UC patients)	4.5 (1.4-14.4)	3.8 (1.1-13.3)
First degree relative with CRC diagnosis	3.2 (1.4-7.6)	3.9 (1.6-9.5)
Post inflammatory polyps	1.1 (0.7-1.9)	1.1 (0.6-2.1)
History of PSC	2.6 (1.4-4.7)	2.5 (1.2-5.0)

PSC, primary sclerosing cholangitis; CRC, colorectal cancer.

DISCUSSION

The aim of this retrospective study was to assess whether the risk-stratified approaches of the updated AGA or BSG guidelines for surveillance were more effective in terms of colonoscopic workload and neoplasia yield. The new BSG guidelines were found to only moderately discriminate between the three risk groups with regard to the overall incidence of CAN (3.6%, 6.9% and 10.8% for the low, medium and high-risk groups, respectively). In contrast, the overall yield of CAN in the low-risk group of the AGA guidelines was 5.3% which was significantly lower than the 20.3% for the high-risk group. However, if you compare the predictive power of the risk groups of either guideline using the C-statistic, both guidelines show a similar poor discriminative power with values of 0.55 (BSG) and 0.57 (AGA).

The differences in neoplasia yield come with a cost, however: the risk of CAN in the low-risk group of the AGA guidelines is higher than in the low-risk group of the BSG guidelines (5.3% vs 3.6%). Whether the shorter surveillance intervals of the AGA guideline protects these low-risk

patients from developing advanced neoplasia remains to be studied.

A potential advantage of risk stratification is the avoidance of unnecessary colonoscopies. We found that implementation of the BSG guidelines reduced the colonoscopic workload by 22% as compared to the AGA guidelines. The authors of the new BSG guidelines estimated the percentages of patients in each risk group to be 15%, 30% and 55% for the high-, intermediate- and low-risk group which is more or less in line with our results (20%, 39% and 41%, respectively).[9] One other study also reported that the colonoscopic workload could be reduced by 15% employing the new BSG guidelines.[10]

However, these differences in colonoscopic workload critically depend on arbitrarily chosen surveillance intervals. For example, if a surveillance interval of three years would be applied to the lower risk group of the AGA guidelines, the colonoscopic workload would be 9% lower than the workload associated with the BSG guidelines. Since no prospective data are available comparing different surveillance intervals in colitis patients, stratification as defined by the AGA guidelines with a surveillance interval of three years for the low-risk group might offer the best compromise with regard to identification of CAN and reduction of colonoscopic workload.

Some established risk factors for colitis-associated CRC implemented in the new AGA and BSG guidelines were confirmed in our study. We found a 2.5-fold higher risk of developing CAN in patients with a concomitant diagnosis of PSC, which is in line with earlier reports.[11,12] Patients with a first-degree relative diagnosed with CRC had a higher risk as well, although conflicting data in literature exist on this issue.[13-16] We also confirmed that the absence of histological signs of inflammation is associated with a reduced risk of developing CRC.[17] Several studies have suggested that endoscopic features reflecting longstanding severe inflammation such as post-inflammatory polyps, strictures and active inflammation during surveillance can predict the risk of subsequent development of neoplasia as well.[18,19] Apart from the association between strictures in patients with UC and neoplasia development, we could not confirm this. Strictures in patients with UC are rare, but if present carry a high risk of CRC.[20,21] In our study, nine UC patients developed a stricture of which two developed CRC and one HGD, underscoring the need for close surveillance or even colectomy in these patients. The moderate performance of the BSG guidelines in identifying patients at higher risk of CAN in our study can partially be attributed to its strong dependence on endoscopic parameters. Based on our results, it seems questionable whether these factors should be used to stratify patients for surveillance. Our study has several limitations. Due to the retrospective design, patients in the current study were not screened in accordance with the updated intervals of the BSG and AGA guidelines and therefore the longer intervals of three and five years were not yet implemented. Although the incidence of CAN was used as an endpoint to compare the different risk strata, the

potential drawbacks of a longer surveillance interval such as the occurrence of more advanced neoplasia and interval carcinomas could not be assessed. Furthermore, the presence of known risk factors for IBD-associated CRC such as a diagnosis of PSC or the presence of post-inflammatory polyps could have triggered an increased awareness from the endoscopists which could have overestimated the incidence of CAN in these high-risk patients. However, since we found only a moderate discriminative power for the risk factors used in both guidelines the presence of this bias would only underscore our results.

Despite the fact that more than 1000 patients were included, only 11 patients developed CRC (during 3172 patient-years of follow up). For that reason, we also included colitis-associated LGD and HGD in a composite endpoint. Especially for lesions with LGD, there is no consensus on how colitis-associated lesions can be distinguished from sporadic adenomas, which could have introduced bias. Furthermore, there is considerable interobserver variability among pathologists for the diagnosis of LGD in the setting of colitis which could have introduced bias as well since the pathology slides were not reviewed by an expert panel.[22] We aimed to minimize the interference of sporadic adenomas and carcinomas by reviewing the endoscopic and histological description of each lesion containing dysplasia and excluding all discrete sessile or pedunculated lesions containing adenomatous tissue.

Both the updated AGA and BSG guidelines advocate the use of chromoendoscopy with targeted biopsies because of a superior neoplasia yield over random biopsies.[23] Since most colonoscopies in the current study were performed prior to the publication of these updated guidelines, only 53 colonoscopies (2%) were performed employing chromoendoscopy. Whether chromoendoscopy performs better among high-risk patients as compared to intermediate or low risk patients is currently unknown and therefore we refrain from speculation how chromoendoscopy would have affected the results if employed in the majority of patients. Patients were stratified for their next surveillance interval, but we were unable to incorporate changing surveillance intervals during follow-up due to transient risk factors, particularly inflammation. We believe however, that although some patients will be assigned to other surveillance intervals because of this, the total number of patients with active inflammation will remain approximately stable over time and therefore the influence on colonoscopic workload is probably small.

Due to the retrospective design we relied on the endoscopy reports to determine whether endoscopic risk factors such as active inflammation or post-inflammatory polyps were present. The fact that endoscopists might interpret the endoscopic findings differently could have resulted in an over or underestimation of the presence of these factors. Furthermore, well-defined and validated endoscopic and histologic scores reliably reflecting the severity of inflammation were not used.

We included both patients with ulcerative colitis and Crohn's colitis in the current study. Although several studies indicate that the risk of CRC is similar when disease extent and duration are comparable most studies reporting on risk factors for developing CRC only include UC patients.[24-26] It might well be that risk factors for CRC are different in patients with Crohn's colitis which could have affected our results. However, in the univariate and multivariate analysis of individual risk factors, the type of IBD was not associated with the development of CAN.

In conclusion, this study shows the clinical consequences if the new AGA or BSG guidelines are applied to a large cohort of IBD patients undergoing surveillance. Although the longer surveillance intervals of the new BSG guidelines reduce the colonoscopic workload considerably compared to the AGA guidelines, the risk strata as defined in the AGA guidelines are superior in distinguishing patients at high and low risk of CAN. Furthermore, if a three year surveillance interval is applied to the lower risk group of the AGA guidelines, the workload is 9% lower compared to the BSG guidelines. Whether the lower incidence of CAN in the low-risk groups of both guidelines justifies longer surveillance intervals is presently unknown and should be the focus of future studies.

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Chapter 5

Adenomas in patients with inflammatory bowel disease are associated with an increased risk of advanced neoplasia

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Published in Inflammatory Bowel Diseases

ABSTRACT

Background

It is still unclear whether IBD patients with adenomas have a higher risk of developing high-grade dysplasia (HGD) or colorectal cancer (CRC) than non-IBD patients with sporadic adenomas. We compared the risk of advanced neoplasia (AN, defined as HGD or CRC) in IBD patients with adenomas to IBD patients without adenomas and patients without IBD with adenomas.

Methods

IBD patients with a histological adenoma diagnosis (IBD + adenoma), age-matched IBD patients without adenoma (IBD-nonadenoma) and adenoma patients without IBD (nonIBD + adenoma) were enrolled in this study. Medical charts were reviewed for adenoma characteristics and development of AN. The endoscopic appearance of the adenomas was characterized as *typical* (solitary sessile or pedunculated) or *atypical* (all other descriptions).

Results

A total of 110 IBD + adenoma patients, 123 IBD-nonadenoma patients and 179 nonIBD + adenoma patients were included. Mean duration of follow-up was 88 months (SD ±41). The 5-year cumulative risks of AN were 11%, 3% and 5% in IBD + adenoma, IBD-nonadenoma and nonIBD + adenoma patients, respectively ($p<0.01$). In IBD patients atypical adenomas were associated with a higher 5-year cumulative risk of AN compared to IBD patients with typical adenomas (18% versus 7%, $p=0.03$).

Conclusions

IBD patients with a histological diagnosis of adenoma have a higher risk of developing AN than adenoma patients without IBD and IBD patients without adenomas. The presence of atypical adenomas in particular was associated with this increased risk, although patients with typical adenomas were found to carry an additional risk as well.

INTRODUCTION

It is commonly accepted that both Crohn's colitis and ulcerative colitis (UC) are associated with an increased risk of colorectal cancer (CRC).[1] The cumulative risk was found to be 18% after 30 years of disease duration in patients with UC and similar risks have been reported in patients with Crohn's colitis.[2,3] Colitis-associated CRC is thought to be preceded by dysplasia, which can be found in flat mucosa or in endoscopically visible lesions. For the latter the term dysplasia-associated lesion or mass (DALM) was coined in 1981.[4] DALMs are heterogenous in their endoscopic appearance and are therefore subdivided in adenoma-like and non-adenoma-like lesions. The non-adenoma-like subtype refers to all irregular, diffuse masses or plaque lesions that generally cannot be removed by endoscopic resection. These lesions were reported to be frequently accompanied by synchronous malignancy and guidelines therefore generally recommend colectomy, although data supporting these guidelines are limited.[5,6] The adenoma-like DALM refers to all discrete, either pedunculated or sessile polyps which resemble sporadic adenomas in patients without IBD. Previous studies reported that CRC risk is low in these patients and that polypectomy with regular surveillance is an adequate treatment strategy.[7,8] Although these initial studies were based on small patient numbers with limited follow-up, two larger studies published more recently seemed to confirm these data.[9,10] However, little is known about the additional risk of adenomas in IBD patients as compared to IBD patients without an adenoma and patients with an adenoma but without IBD.

The aim of this study was therefore to describe the risk of subsequent colorectal neoplasia in a large cohort of IBD patients with an adenoma, and to compare this risk to that in IBD subjects without adenomas as well as non-IBD patients with adenomas.

METHODS

Patients

The nationwide pathology archive (PALGA) that contains all pathology reports from the Netherlands dating back to 1971, was used to identify three cohorts of patients: 1. IBD patients with an adenoma (IBD + adenoma); 2. IBD patients without an adenoma (IBD-nonadenoma) and 3. subjects with an adenoma but without IBD (nonIBD + adenoma). A PALGA search was performed in seven university medical centres for the period between 1995 and 2005. IBD-nonadenoma patients were matched to IBD + adenoma patients for age at inclusion and centre. Year of inclusion was matched to the year of adenoma diagnosis in the IBD + adenoma patients. The latter was performed in order to obtain an equal follow-up time between these groups. Exclusion criteria were a history of any dysplasia (low-grade dysplasia (LGD) or

high-grade dysplasia (HGD)) or CRC, a diagnosis of HGD or CRC at the moment of adenoma diagnosis, a history of (sub)total colectomy, no endoscopic or surgical follow-up and incomplete or missing data. Furthermore, patients with Crohn's disease without involvement of the colon were excluded.

Data collection

Demographic and clinical data were collected from medical charts for all three cohorts. For the IBD + adenoma and IBD-nonadenoma cohorts, clinical data included date of IBD diagnosis, type of IBD, extent of IBD, medication use and the presence of primary sclerosing cholangitis. Disease extent was defined as the maximum extent according to either histology or endoscopy reports. In UC and indeterminate colitis (IC) or IBD-unclassified (IBD-U) patients disease extent was defined as either left-sided or extensive (inflammation distal or proximal to the splenic flexure, respectively). In patients with Crohn's colitis, involvement of 3 or more anatomical parts of the colon was considered extensive disease, whereas involvement of 1 or 2 sections was considered limited disease. In all three cohorts a family history of CRC was documented. For the IBD + adenoma and nonIBD + adenoma cohorts, histopathology and endoscopy reports were reviewed to identify the date of first adenoma diagnosis and to collect information about size, location within or outside an area of previous inflammation and endoscopic appearance. We selected patients based on their histological adenoma diagnosis. However, in the endoscopy reports we encountered a wide variety of endoscopic descriptions of adenomas. Therefore, for practical purposes, adenomas were classified as either 'typical' or 'atypical'. Typical adenomas included all lesions described as discrete solitary, sessile or pedunculated polyps resembling sporadic adenomas. All other endoscopic lesions, including adenomatous fields (i.e. areas of multiple, clustered polyps), lesions with an irregular surface and lesions endoscopically described as post-inflammatory polyps (but histologically classified as adenoma) were characterized as atypical adenomas. Dysplasia was classified as either low-grade or high-grade, according to the criteria and definitions articulated by Riddell et al.[11]

Follow-up

Histopathology and endoscopy reports were reviewed to detect whether patients developed LGD, HGD or CRC during follow-up. Advanced neoplasia (AN) was defined as a finding of HGD or CRC. Duration of follow-up was measured in months and defined as time from the first adenoma diagnosis (IBD + adenoma and nonIBD + adenoma cohorts) or from the moment of inclusion in the study (IBD-nonadenoma cohort) to one of the following endpoints: 1. end of follow-up 2. end of study period (1st of December, 2010), 3. death, or 4. subtotal or total colectomy.

Statistical analysis

All analyses were restricted to the period beyond the first 6 months of follow-up to exclude patients with prevalent AN.

Baseline characteristics were analyzed with standard descriptive statistics and compared between the three groups. Continuous variables were analyzed using ANOVA or Kruskall-Wallis analysis, where appropriate. Categorical variables were analyzed using Pearson's chi-squared or Fisher's exact test, where appropriate. Five-year cumulative incidences of AN were calculated using Kaplan-Meier survival analysis and comparisons between groups were made using log-rank testing. Patients who did not develop AN during follow-up were censored at the moment of last colonoscopy or colectomy. Factors associated with the development of AN during follow-up in IBD patients (IBD + adenoma and IBD-nonadenoma patients) were assessed in a Cox proportional hazard model. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS 15.0 for Windows.

Ethical considerations

This study was carried out with the approval of and in accordance with the ethical guidelines of the research review committee of our institution.[12]

RESULTS

Patients

Our search yielded 617 IBD + adenoma patients, 472 IBD-nonadenoma patients and 902 nonIBD + adenoma patients. Review of medical charts, endoscopy, pathology and surgery reports yielded 110 IBD + adenoma, 179 nonIBD + adenoma and 123 IBD-nonadenoma patients eligible for enrolment. The reasons for exclusion are shown in the flowchart (Figure 1). Clinical characteristics of the three patient groups are given in Table 1.

Adenoma characteristics

A total of 216 adenomas were identified in 179 nonIBD + adenoma patients and 133 adenomas in 110 IBD + adenoma patients. Adenoma characteristics are given in Table 2. Adenomas were characterized as typical in 82 (75%) and atypical in 28 IBD + adenoma patients (25%). The latter consisted of 15 lesions endoscopically classified as post-inflammatory polyps (54%) and 13 non-adenoma-like lesions (46%). In nonIBD + adenoma patients 8 adenomas (4%) were characterized as atypical and 171 (96%) as typical.

Polypectomy was performed in 146 nonIBD + adenoma patients (82%) and in 68 IBD + ade-

noma patients (62%) (p<0.01). Median adenoma size was 5 mm (range 2 – 20) in the IBD + adenoma cohort and 6 mm (range 1 – 65) in the nonIBD + adenoma cohort (p=0.11). There were no significant differences between IBD + adenoma and nonIBD + adenoma patients regarding adenoma location or architecture. In 21 IBD + adenoma patients (19%) adenomas were located outside the maximum endoscopic or histological extent of inflammation.

Figure 1: Flow chart of patient selection and development of neoplasia during follow-up.

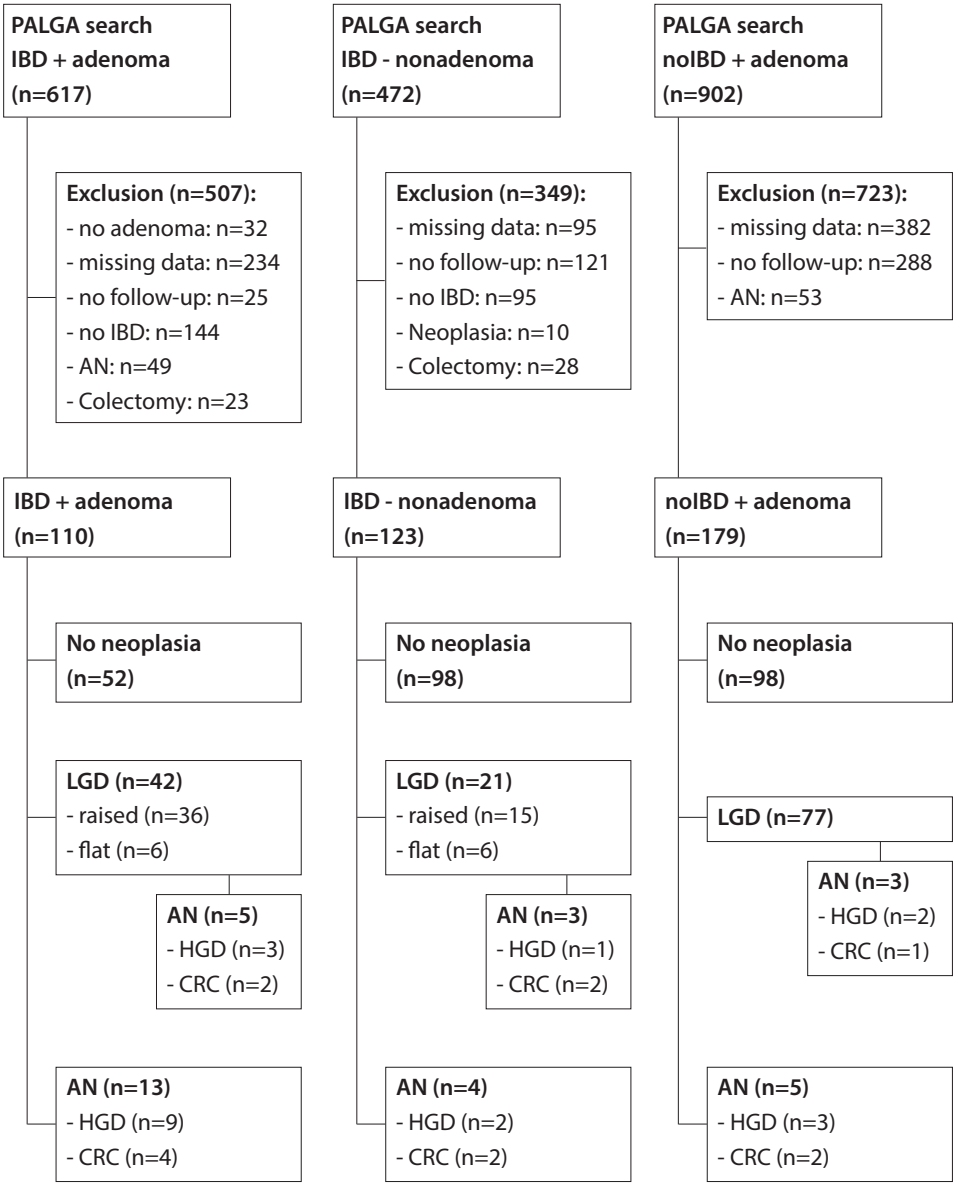


Table 1. Baseline characteristics

	IBD + adenoma N = 110 (%)	IBD- nonadenoma N = 123 (%)	nonIBD + adenoma N = 179 (%)
IBD diagnosis - Ulcerative colitis	73 (66)	53 (43)	NA
Proximal to splenic flexure	41 (56)	31 (58)	
Distal to splenic flexure	31 (43)	22 (42)	
Unknown	1 (1)	0 (0)	
Crohn's disease	32 (29)	52 (42)	NA
Extensive disease	14 (44)	32 (62)	
Limited disease	17 (53)	20 (38)	
Unknown	1 (3)	0 (0)	
Indeterminate colitis / IBDU	5 (5)	18 (15)	
Proximal to splenic flexure	3 (60)	8 (44)	
Distal to splenic flexure	1 (20)	10 (56)	
Unknown	1 (20)	0 (0)	
Age at IBD diagnosis, yr (mean ± SD)	42 (17)	39 (15)	NA
Age at initial adenoma diagnosis / start follow-up, yr (mean ± SD)	56 (12)	53 (12)	59 (12)
Duration of IBD, yr (mean ± SD)	14 (13)	14 (12)	NA
Gender – male	64 (58)	67 (55)	84 (47)
History of PSC	3 (3)	6 (5)	0 (0)
Family history of CRC[a]	4 (4)	10 (8)	43 (24)
Medication use			
5-ASA > 6 months	97 (88)	105 (88)	NA
Thiopurines > 6 months	32 (29)	40 (33)	NA
Infliximab	7 (6)	16 (13)	NA
Duration of follow-up, months (mean ± SD)	84 (43)	96 (40)	85 (40)
Number of follow-up colonoscopies (median, range)	3 [1-15]	3 [0-11][b]	2 [1-12]
Partial or total colon resection during follow-up	18 (16)	14 (11)	7 (4)
Indication - neoplasia	14 (77)	5 (36)	7 (100)
Indication - IBD	4 (23)	9 (64)	0 (0)

IBD, inflammatory bowel disease; IBDU, IBD-unspecified; PSC, primary sclerosing cholangitis; SD, standard deviation.[a] Unknown in 188 patients. [b] One patient in the IBD-nonadenoma cohort underwent colectomy during follow-up but no colonoscopy

Table 2: Characteristics of adenomas in patients with and without IBD

	IBD + adenoma N = 110 (%)	**nonIBD + Adenoma N = 179 (%)**	**p-value**
Treatment of adenoma			
Biopsy	41 (37)	33 (18)	<0.01
Endoscopic removal	68 (62)	146 (82)	
Partial or total colon resection	1 (1)	0 (0)	
Endoscopic appearance			
Typical polyp	82 (75)	171 (96)	
Atypical polyp	28 (25)	8 (4)	NA
Post-inflammatory polyp	15 (54)	NA	
Non adenoma-like	13 (46)	8 (100)	
Adenoma located in area of IBD	89 (81)	NA	
Number of adenomas	133 (%)	216 (%)	
Location of adenoma			
Rectum	19 (14)	48 (22)	
Sigmoid	55 (41)	91 (42)	
Descending colon	10 (8)	15 (7)	0.21
Transverse colon	24 (18)	19 (9)	
Ascending colon	13 (10)	17 (8)	
Cecum	11 (8)	21 (10)	
Unknown	1 (1)	5 (2)	
Architecture			
Tubular	76 (57)	131 (61)	
Tubulovillous	39 (29)	70 (32)	0.22
Villous	8 (6)	4 (2)	
Serrated	1 (1)	7 (3)	
Other	9 (7)	4 (2)	
Size of adenoma, mm (median, range)	5 [2-20]	6 [1-65]	0.11

IBD, inflammatory bowel disease

Development of neoplasia in IBD + adenoma patients during follow-up

In 36 IBD + adenoma patients (33%) adenomas were detected during follow-up, which were endoscopically classified as typical in 29 (81%) and atypical in seven patients (19%). Four other patients (4%) developed flat LGD and in two patients (2%) both flat and raised dysplasia was detected.

AN was diagnosed in 18 IBD + adenoma patients (16%) after a median follow-up of 53 months (range 7 - 86) (Figure 1). Twelve patients developed HGD and six developed CRC, of which three were preceded by HGD. In 10 patients HGD was detected in typical adenomas, which were treated by polypectomy in seven and by subtotal colectomy in two patients. In one of these patients, CRC was detected in the resection specimen. One patient with HGD in a large typical adenoma was not treated with endoscopic or surgical resection due to advanced age. HGD was diagnosed in atypical lesions in three patients and in flat mucosa in two. Of these five patients, three were treated by colectomy. HGD was confirmed in the colectomy specimen of one patient while in the other two patients CRC was detected. In the remaining two patients no polypectomy or colectomy was performed due to advanced age and because the diagnosis of HGD could not be confirmed during follow-up colonoscopies. In 89% of patients AN was located in the colonic segment where the baseline adenoma was situated. Two patients, of whom one was originally treated by polypectomy and one was not, developed AN in another colonic segment.

The 5-year cumulative incidence of AN in the IBD + adenoma cohort was 11% (Figure 2). This was similar in patients treated with polypectomy and patients in whom the adenoma was only biopsied (11% versus 10% respectively, p=0.99). Patients with an atypical adenoma at baseline had a higher risk of developing AN compared to patients with a typical adenoma (Figure 3). The 5-year cumulative incidence of AN was not different between patients with an adenoma located inside and patients with an adenoma outside an area of inflammation (10% and 19% respectively, p=0.91). No differences were found between CD and UC patients regarding development of AN (p=0.11).

Figure 2. Kaplan-Meier curve comparing the development of the composite endpoint advanced neoplasia (HGD and CRC, Figure 2a) and CRC alone (Figure 2b) between the three patient groups (IBD + adenoma, nonIBD + adenoma and IBD-nonadenoma). Vertical lines represent events of advanced neoplasia (p <0.01, IBD + adenoma versus nonIBD + adenoma and IBD + adenoma versus IBD-nonadenoma, log-rank test)

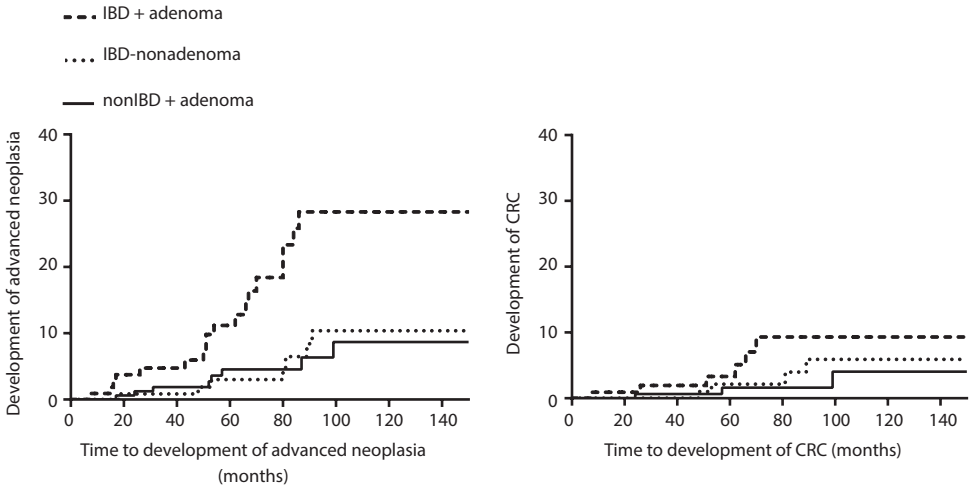
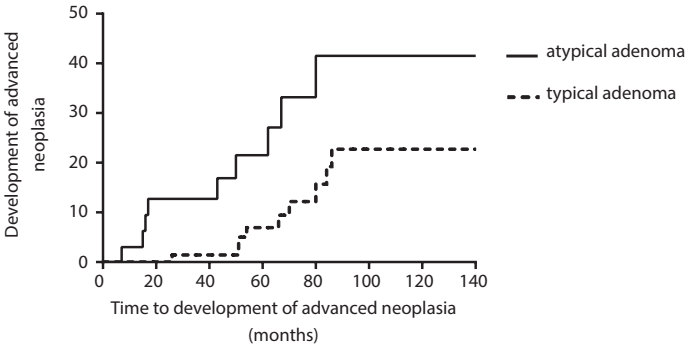


Figure 3. Kaplan Meier curve comparing the development of advanced neoplasia between IBD patients with typical and atypical adenomas (p=0.02, log-rank test).



Development of neoplasia in IBD-nonadenoma patients during follow-up

Within the IBD-nonadenoma cohort 15 patients (12%) developed adenomas during follow-up, which were endoscopically classified as typical adenomas in ten patients and atypical adenomas in five. Two patients developed flat LGD and four developed both flat and raised dysplasia. A total of seven patients (7%) developed AN after a median duration of follow-up of 80 months

(range 18 – 91 months, Figure 1). Three patients developed HGD in a typical adenoma. Of these, two were treated by colectomy and one by polypectomy. None of these patients developed CRC during follow-up. Four patients developed CRC. One of these developed CRC 4 years after an endoscopically resected flat adenoma whereas three developed CRC without evidence of prior LGD or HGD. The 5-year cumulative incidence of AN was 3% in the IBD-nonadenoma cohort (Figure 2). This was significantly lower compared to the IBD + adenoma cohort ($p<0.01$). No differences were found between CD and UC patients regarding development of AN ($p=0.12$). The 5-year cumulative incidence of CRC alone was not significantly different between the IBD-nonadenoma and IBD + adenoma groups (2% vs 4% respectively, $p=0.23$, Figure 2b).

Factors associated with development of AN

The presence of an adenoma in patients with IBD was associated with an increased risk of developing AN during follow-up (HR 3.6, 95% CI 1.5 – 8.7) (Table 3). In the multivariate analysis, this effect remained borderline significant after adjustment for duration and type of IBD, extent of inflammation, age, gender, concomitant diagnosis of PSC and medication use (HR 2.8, 95% CI 1.0 – 8.2). A diagnosis of UC (compared to CD) was associated with an increased risk of developing AN as well, (unadjusted HR 6.1, 95% CI 1.4 – 26.4) which remained significant in the multivariate analysis (adjusted HR 4.5, 95% CI 1.0 – 25.4). Medication use, including 5-ASA and thiopurines, was not associated with the development of AN.

Table 3. Cox proportional hazard analysis of the association between the presence of an adenoma at baseline and the incidence of advanced neoplasia in the IBD patient groups (IBD + adenoma and IBD - nonadenoma).

	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Presence of adenoma	3.6 (1.5-8.7)	2.8 (1.0-8.2)
Male gender	1.1 (0.5-2.4)	0.8 (0.3-2.1)
Ulcerative colitis (vs. Crohn's disease)	6.1 (1.4-26.4)	5.1 (1.0-25.4)
Extensive colitis	1.5 (0.6-3.5)	1.7 (0.6-4.7)
5-ASA use	0.8 (0.3-2.3)	0.8 (0.2-5.5)
Thiopurine use	0.8 (0.3-1.9)	1.1 (0.4-3.5)
History of PSC	2.0 (0.5-8.4)	3.9 (0.7-21.3)
Duration of IBD, years	1.0 (0.9-1.0)	1.0 (0.9-1.0)

PSC, primary sclerosing cholangitis; IBD, inflammatory bowel disease

Development of neoplasia in nonIBD + adenoma patients during follow-up

In 77 nonIBD + adenoma patients (42%) adenomas were detected during follow-up. A total of eight patients (4%) developed AN after a median follow-up of 53 months (range 17-99 months, Figure 1). In five patients HGD was detected in an adenoma that was treated endoscopically in all five patients. None of these patients developed CRC during follow-up. Three nonIBD + adenoma patients developed CRC, which were all treated by surgical resection.

The 5-year cumulative incidence of AN in the nonIBD + adenoma cohort was 5% (Figure 2). This was significantly lower compared to the IBD + adenoma cohort ($p<0.01$), as well as to the subset of IBD + adenoma patients with a typical adenoma resembling sporadic adenomas ($p=0.03$). The 5-year cumulative incidence of CRC alone in the nonIBD + adenoma cohort did not differ from the IBD + adenoma cohort (1% vs 3% respectively, $p=0.06$, Figure 2b).

DISCUSSION

This study demonstrates an increased risk of AN in IBD patients with an adenoma, when compared to non-IBD patients with adenomas and IBD patients without adenomas. Notably, atypical as well as typical adenomas in patients with IBD were found to be associated with an increased risk of AN. This finding is in contrast with most previously published studies, which consistently reported a low risk of AN in IBD patients with polypoid dysplasia.[8,10,13,14]

The high incidence of AN among IBD + adenoma patients during follow-up in our study was mainly caused by the development of HGD rather than the development of CRC. HGD developed in 12% of IBD + adenoma patients as compared to 2% and 3% of IBD-nonadenoma and nonIBD + adenoma patients, whereas CRC developed in 6% of IBD + adenoma patients compared to 3% and 2% of IBD-nonadenoma and nonIBD + adenoma patients, respectively. Although previous studies primarily focused on CRC incidence, the reported incidences of HGD after a diagnosis of adenoma were low in these studies and varied between 0% and 5%.[8-10,15] Since HGD will undoubtedly progress to CRC when left untreated, we opted to use a composite endpoint including both HGD and CRC.[16-18] Furthermore, we report a substantially higher CRC incidence in IBD + adenoma patients than reported in several previous studies.[8,10,13] One might assume that the inclusion of both patients treated by polypectomy and patients in whom adenomas were not endoscopically removed accounts for the higher risk of AN in our cohort. Eighty-one percent of all nonIBD + adenoma patients were treated by polypectomy, compared to only 62% of IBD + adenoma patients. An explanation for the relative low polypectomy rate in the IBD + adenoma cohort might be that post-inflammatory-like and small flat lesions are more frequently encountered and not deemed serious enough for polypectomy.

Our rates are in line with recent data from Vieth et al.,[9] who found a polypectomy rate of 59% in IBD patients with adenomas, although we could not confirm the higher incidence of neoplasia and CRC among patients in whom no polypectomy was performed as reported in that study. It is therefore conceivable that AN developed either as a result of field cancerization or due to residual dysplastic tissue.

Another possible explanation for the high incidence of AN might be the design of our study. We selected cases using a pathology database, whereas in previous studies selection was based on the endoscopic identification of solitary polyps.[8,10,13] Therefore, our study comprised a heterogeneous group of endoscopic lesions including a large subgroup of "non-adenoma-like" lesions, which have previously been associated with a high risk of synchronous CRC.[4,19-21] Current guidelines therefore generally recommend colectomy for these types of lesions.[5,6] In our IBD + adenoma cohort, 25% of patients had an atypical adenoma, including both non-adenoma-like lesions and lesions considered to be post-inflammatory polyps during endoscopy. The cumulative incidence of AN was substantially higher in these patients: eight patients with an atypical adenoma (29%) developed AN compared to ten patients (12%) with a typical adenoma. Interestingly, in 15 patients histologically diagnosed adenomas were endoscopically characterized as post-inflammatory polyps. Of these, 30 % developed AN during follow-up, which highlights the difficulty of differentiation between raised dysplasia and post-inflammatory polyps. Inexperience of the endoscopists who assessed the types of polyps may have contributed to this misclassification. Another explanation may be the fact that post-inflammatory polyps are proxies for longstanding and severe inflammation and may therefore be associated with an increased risk of CRC.[22,23]

Of note, IBD + adenoma patients with typical adenomas displayed a significantly increased 5-year cumulative incidence of advanced neoplasia as well: 6% compared to 5% in nonIBD + adenoma patients (p=0.03 log rank test). This conflicts with data from previous studies that reported no difference in development of subsequent neoplasia between these patient groups.[7,13] Our results stress the importance of complete removal and close follow-up of either type of adenoma.

The IBD + adenoma cohort contained patients with adenomas detected both inside and outside colonic areas (previously) involved in inflammation. Since by definition IBD-associated dysplasia only develops in areas of chronic inflammation, one should classify adenomas outside an area of previous inflammation as sporadic. Remarkably, the incidence of AN was not different between IBD patients with adenomas detected within or outside an area of previous inflammation.

The comparison of the IBD + adenoma and IBD-nonadenoma cohorts enabled us to assess the additional risk of adenomas in patients with colitis. IBD + adenoma patients had a higher

risk of AN compared to IBD-nonadenoma patients. The presence of an adenoma seemed an independent predictor of AN when corrected for several known risk factors of IBD-associated neoplasia, although with borderline significance (HR 3.0, 95% CI 1.0 – 8.5).

Our study has some limitations. First, due to the retrospective design of the study we relied on the descriptions provided in endoscopy reports with regard to endoscopic characteristics and treatment of the adenomas. Second, IBD + adenoma patients and IBD-nonadenoma patients were solely matched on age at inclusion, centre and date of inclusion according to the date of adenoma diagnosis in the IBD + adenoma cohort. We do not think that this introduced important bias, though, since these cohorts were quite well-matched with regard to duration and extent of IBD. Third, a signficant proportion of patients were excluded due to the lack of follow-up or missing endoscopy or pathology reports. Since patients in the nonIBD + adenoma and IBD-nonadenoma cohort were more frequently excluded than patients in the IBD + adenoma cohort, this might have resulted in a higher risk of AN in these two cohorts, thereby introducing a selection bias. Obviously, this would only strengthen our conclusion that the risk of AN is increased in the IBD + adenoma patients. At last, although patients underwent a complete colonoscopy at inclusion in this study and at least one colonoscopy or a colectomy during follow-up, surveillance colonoscopies with random biopsy sampling were performed in only 52% of IBD + adenoma patients and 55% of IBD-nonadenoma patients. This might have resulted in an underestimation of the presence of AN. However, since most cases of AN can be identified endoscopically, we do not feel that this had a major influence on our results.

In conclusion, this study shows that IBD patients with a histological diagnosis of an adenoma have an increased risk of developing AN compared to nonIBD + adenoma patients and IBD-nonadenoma patients. The presence of atypical adenomas in particular was associated with this increased risk, although patients with typical adenomas were found to carry an additional risk as well. Thus, complete removal of adenomas and subsequent strict surveillance is warranted in IBD patients with atypical as well as typical colonic adenomas.

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Chapter 5

Chapter 6

Low inter-observer agreement among endoscopists in differentiating dysplastic from non-dysplastic lesions encountered during colitis surveillance

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Submitted to Journal of Crohn's and colitis

ABSTRACT

Introduction

During endoscopic surveillance in patients with longstanding colitis a variety of lesions can be encountered. Differentiation between dysplastic and non-dysplastic lesions can be challenging. The accuracy of visual endoscopic differentiation and inter-observer agreement (IOA) has never been objectified.

Methods

We assessed the accuracy of expert and non-expert endoscopists in differentiating (low-grade) dysplastic from non-dysplastic lesions and the IOA among and between them. An online questionnaire was constructed containing 30 cases including a short medical history and an endoscopic image of a lesion found during surveillance employing chromoendoscopy..

Results

A total of 17 endoscopists, 8 experts and 9 non-experts, assessed all 30 cases. The overall sensitivity and specificity for correctly identifying dysplasia were 73.8% (95% CI 62.1-85.4) and 53.8% (95% CI 42.6-64.7), respectively. Experts showed a sensitivity of 76.0% (95% CI 63.3-88.6) versus 71.8% (95% CI 58.5-85.1, p=0.434) for non-experts ,the specificity 61.0% (95% CI 49.3-72.7) versus 47.1% (95% CI 34.6-59.5, p=0.008) . The overall IOA in differentiating between dysplastic and non-dysplastic lesions was fair 0.24 (95% CI 0.21-0.27); for experts 0.28 (95% CI 0.21-0.35) and for non-experts 0.22 (95% CI 0.17-0.28). The overall IOA for differentiating between subtypes was fair 0.21 (95% CI 0.20-0.22); for experts 0.19 (95% CI 0.16-0.22) and non-expert 0.23 (95% CI 0.20-0.26).

Conclusion

In this image-based study, both expert and non-expert endoscopists cannot reliably differentiate between dysplastic and non-dysplastic lesions. This emphasizes that all lesions encountered during colitis surveillance with a slight suspicion of containing dysplasia should be removed and sent for pathological assessment.

INTRODUCTION

Patients with inflammatory bowel disease (IBD) are at increased risk of developing colorectal cancer (CRC).[1-5] Since the risk of colitis-associated CRC increases with a longer disease duration and greater disease extent, [6,7] current guidelines recommend colonoscopic surveillance in all IBD patients with longstanding extensive colitis.[8-10]

During surveillance colonoscopy in IBD patients, a variety of dysplastic and non-dysplastic lesions can be found. Dysplastic lesions can be classified as either sporadic adenomas, i.e. not related to the inflammation, or colitis-associated dysplasia, also known as dysplasia associated lesion or mass (DALM). Differentiation between these entities is challenging, as there are no distinct histological features to confirm the diagnosis. In the past, the risk of synchronous and metachronous colorectal cancer was considered to be very high in patients with lesions classified as DALM and therefore, prophylactic proctocolectomy was advocated. [11] Nowadays there is ongoing debate whether endoscopic resection is justified in some of these cases.

Non-dysplastic lesions, comprising post-inflammatory polyps, lesions caused by scarring or active inflammation, and common non-dysplastic lesions such as hyperplastic polyps can be found as well. This wide variety of lesions renders differentiation between dysplastic and non-dysplastic lesions challenging. Several studies have demonstrated that pan-chromoendoscopy (pCE), employing methylene blue or indigo carmine, improves the detection of dysplastic lesions during IBD surveillance colonoscopies, [12-14] and therefore current guidelines recommend this technique as the preferred method.[9-10] In addition, pCE could also aid in the endoscopic differentiation of lesions found during colonoscopy.[15]

In the current study we aimed to investigate the inter-observer accuracy, agreement and variability between expert and non-expert endoscopists in differentiating dysplastic from non-dysplastic lesions encountered in patients with longstanding colitis undergoing pCE surveillance.

METHODS

An online questionnaire (LimeSurvey, www.limesurvey.com) was constructed containing images of 30 lesions encountered during CRC surveillance in IBD patients. High quality images of lesions were selected from a database containing patients with longstanding ulcerative colitis (UC) and Crohn's disease (CD), enrolled in a surveillance program in two academic centres from 2009 to 2013. Cases were excluded when disease activity was present during surveillance colonoscopy, rated by the performing endoscopist. In all cases, Olympus CF-160 and

CF-180 colonoscopes were used and pCE with either methylene blue 0.1% or indigo-carmine 0.3% was employed. For every case, a histological diagnosis was available to serve as golden standard. One endoscopic image of each lesion was uploaded into the questionnaire without any form of post-processing besides removing the name of the patient from the image and highlighting the lesion with an arrow. In total, 13 lesions containing low-grade dysplasia (LGD) and 17 non-dysplastic lesions were selected. One expert GI-pathologist reassessed all dysplastic lesions available according to Vienna classification.[16]
The cases were numbered, and for each case, two slides were prepared. The first one listed a standardized short medical history (age, gender, type and duration of IBD, disease extent, prior dysplasia, and concomitant diagnosis of primary sclerosing cholangitis) and an endoscopic image of the lesion, the other slide showed the pathological diagnosis.

Participating endoscopists

Twelve consultant gastroenterologists and five fellows in training completed the questionnaire. Eight gastroenterologists were classified as "expert" as they were working in a referral centre and had performed at least 50 IBD surveillance colonoscopies with pCE. The non-expert group consisted of five fellows in training and four endoscopists working in a general hospital. No specific training was given prior to the questionnaire, although a reference to the current IBD-surveillance guideline and practical instructions on the questionnaire were provided.[9]

Assessment

The participants were asked to classify each lesion into seven categories: sporadic adenoma, adenoma-like DALM, non-adenoma-like DALM, inflammation, post-inflammatory polyp, hyperplastic polyp or normal mucosa. The first three types of lesions (sporadic adenoma, adenoma-like DALM and non-adenoma-like DALM) were classified as dysplastic lesions; the remaining four were combined as non-dysplastic. The confidence of each endoscopic diagnosis was assessed by asking the participants to rate the certainty of each diagnosis from 1 to 5, where 1 was 'very uncertain' and 5 'very certain'.
After the first assessment of each lesion, the histopathological diagnosis of the lesion was provided to the participant. As the pathology report of the lesion could play a role in the subdifferentiation of lesions by the endoscopist, participants were asked to classify all the lesions again after the histopathological diagnosis was given.

Statistical analysis

The sensitivity and specificity for differentiating dysplastic from non-dysplastic lesions were calculated using histopathology of each lesion as reference standard. The sensitivity, specific-

ity, diagnostic accuracy, negative predictive value (NPV) and positive predictive value (PPV) were calculated and compared between experts and non-experts using generalized estimating equation (GEE) with exchangeable correlation structure.[17] GEE was also used for the analysis of the effect of confidence on test accuracy.

The overall inter-observer agreement for the differentiation between dysplastic and non-dysplastic lesions as well as for each subcategory was calculated using Fleiss Kappa, which measures the level of agreement beyond chance. Interpretation of the Kappa values were done according Landis and Koch.[18,19] The inter-observer agreement was also calculated separately for experts and non-experts and compared with Wald statistic, [20] by assuming the Kappa of experts and non-experts had a correlation of 0.3 since they are observing lesions from the same patient group. The post-pathology differentiation between sporadic adenoma, adenoma-like and non-adenoma-like DALM was not compared to a gold standard since there is no uniform definition for these three classifications. The agreement on the differentiation between adenoma-like and non-adenoma-like DALM was calculated based on the classification given after the pathological diagnosis of the lesion. The two dependent kappa's were compared according to Donner.[20]

RESULTS

Participants were able to correctly classify lesions as dysplastic with a sensitivity of 73.8% (95% CI 66.1-85.4), a specificity of 53.8% (95% CI 42.6-64.7) and an accuracy of 62.4% (95% CI 53.8-71.1), shown in table 1. The overall inter-observer agreement for the differentiation between dysplastic and non-dysplastic lesions was fair with a Kappa of 0.24 (95% CI 0.21-0.27).

Table 1: Inter-observer agreement and diagnostic accuracy for the differentiation between dysplastic and non-dysplastic lesions

	Overall (n=17)	**Experts (n=8)**	**Non-experts (n=9)**	**P-value**
Inter-observer agreement	0.24 (0.21 – 0.27)	0.28 (0.21 – 0.35)	0.22 (0.17 – 0.28)	0.163
Sensitivity (%)	73.8 (62.1 – 85.4)	76.0 (63.3 – 88.6)	71.8 (58.5 – 85.1)	0.434
Specificity (%)	53.8 (42.6 – 64.7)	61.0 (49.3 – 72.7)	47.1 (34.6 – 59.5)	0.008
Accuracy (%)	62.4 (53.6 – 71.1)	67.5 (58.5 – 76.5)	57.8 (47.6 – 67.9)	0.010
NPV (%)	73.0 (68.1– 77.9)	76.8 (72.2 – 81.4)	68.5 (60.9 – 76.1)	0.064
PPV (%)	54.3 (49.6 – 59.1)	59.8 (51.5 – 68.2)	50.8 (46.0 – 55.7)	0.109

NPV = negative predictive value, *PPV* = positive predictive value

Comparison between experts and non-experts

The sensitivity for identifying lesions containing dysplasia was 76.0% for experts, which was not significantly different from the non-experts (71.8%, p=0.50), but experts had a significantly higher specificity (61.0% versus 47.1% respectively, p=0.008, table 1).

The inter-observer agreement for the differentiation between dysplastic and non-dysplastic lesions was fair, for experts as well as non-experts, 0.28 (95% CI 0.21-0.35) and 0.22 (95% CI 0.17-0.28) respectively (p=0.163).

The overall inter-observer agreement for each subcategory is shown in table 2. The agreement was highest for the classifications post-inflammatory polyp, hyperplastic polyp and sporadic adenoma (all κ=0.26) and lowest for non-adenoma-like DALM (κ=0.12). Experts showed a significantly higher agreement for the category 'post-inflammatory polyp', while non-experts showed a significantly higher agreement for the categories 'normal mucosa' and 'non-adenoma-like DALM'. (table 2).

Table 2: Inter-observer agreement *before* pathological diagnosis was given

	Overall (n=17)	**Experts (n=8)**	**Non-experts (n=9)**	**P-value**
Overall	0.21 (0.20 – 0.22)	0.19 (0.16 – 0.22)	0.23 (0.20 – 0.26)	0.033
Normal mucosa	0.20 (0.17 – 0.23)	0.08 (0.01 – 0.14)	0.28 (0.22 – 0.34)	0.000
Inflammation	0.19 (0.16 – 0.22)	0.18 (0.11 – 0.24)	0.15 (0.09 – 0.21)	0.516
Post-inflammatory polyp	0.26 (0.23 – 0.29)	0.36 (0.29 – 0.43)	0.19 (0.13 – 0.25)	0.000
Hyperplastic polyp	0.26 (0.23 – 0.29)	0.27 (0.20 – 0.34)	0.27 (0.21 – 0.32)	0.876
Sporadic adenoma	0.26 (0.23 – 0.29)	0.22 (0.15 – 0.29)	0.28 (0.22 – 0.34)	0.126
Adenoma-like DALM	0.17 (0.14 – 0.20)	0.14 (0.07 – 0.20)	0.20 (0.14 – 0.26)	0.113
Non-adenoma-like DALM	0.12 (0.09 – 0.16)	0.10 (0.03 – 0.17)	0.19 (0.13 – 0.25)	0.016

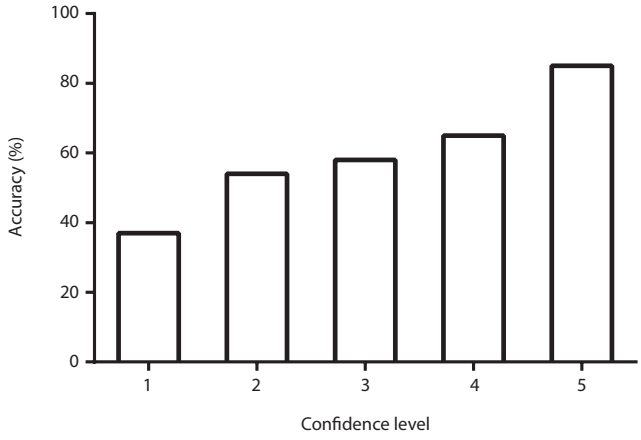
Non-dysplastic: Normal mucosa, inflammation, post-inflammatory polyps, hyperplastic polyp-
Dysplastic: Sporadic adenoma, adenoma-like DALM, non-adenoma-like DALM

Confidence level

For every diagnosis the observers were asked for confidence level of their endoscopic classification, on a scale from 1 to 5. The overall confidence level was 3.45 (95% CI 3.33-3.57). For experts this was significantly higher at 3.59 (95% CI 3.46-3.72) than for non-experts (3.33, 95% CI 3.19-3.47) (p=0.001). Of all diagnoses made, 42.9% (219 out of 510) were made with a confidence level 4 and 10.4% (53 out of 510) with a confidence level 5. The relation between the confidence level and accuracy of differentiating between dysplastic and non-dysplastic lesions for both

experts and non-experts is shown in figure 3. For the lowest confidence level the accuracy was 36.8% and for the highest confidence level 84.9%. The result of the logistic regression of confidence level on test accuracy showed that for one unit increase in the level of confidence, a 35.7% increase in the odds of the test result being accurate is reached.

Figure 3: Relation between accuracy and confidence level differentiating between dysplastic and non-dysplastic lesions for both experts and non-experts



Classification after histopathologic diagnosis

After providing the histopathology report to the participants, the overall inter-observer agreement for the categories sporadic adenoma, adenoma-like DALM and non-adenoma-like DALM assessed for the 13 dysplastic lesions were 0.37, 0.20 and 0.15 respectively (table 3). Only a significant difference between experts and non-experts was found for the category 'non-adenoma-like DALM'.

Table 3: Inter-observer agreement for classifying lesions containing dysplasia (n=13) as adenoma-like or non-adenoma like DALM *after* histopathological diagnosis was provided

	Overall (n=17)	**Experts (n=8)**	**Non-experts (n=9)**	**P-value**
Sporadic adenoma	0.37 (0.33 – 0.42)	0.32 (0.22 – 0.42)	0.41 (0.32 – 0.50)	0.147
Adenoma-like DALM	0.20 (0.15 – 0.24)	0.17 (0.07 – 0.28)	0.26 (0.17 – 0.35)	0.119
Non-adenoma-like DALM	0.15 (0.10 – 0.19)	0.03 (-0.08 – 0.13)	0.19 (0.10 – 0.28)	0.007

DISCUSSION

We demonstrate that, during CRC surveillance in IBD patients, expert as well as non-expert endoscopists differentiate dysplastic from non-dysplastic lesions with an acceptable overall sensitivity of 73.8%. The overall specificity was poor at 53.8%, however. Experts performed significantly better than the non-experts. The inter-observer agreement for the different subtypes of dysplastic and non-dysplastic lesions was low, for both experts and non-experts.

In a study on IBD-surveillance published in 2007, the ability of endoscopists to distinguish adenoma-like from non-adenoma-like DALMs without the use of pCE was assessed.[18] In that study, experts showed a sensitivity of 68% for identifying adenoma-like DALMs, 75% for non-adenoma-like DALMs, and 82% for inflammatory polyps. For non-expert endoscopists accuracy was significantly lower. In our study, we did not assess the sensitivity for differentiation between adenoma-like and non-adenoma-like DALMs, as there is no uniform definition for diagnosis of these lesions. However, results seem comparable and experience seems to play an important role in the accuracy of the optical diagnosis of dysplastic lesions during IBD surveillance colonoscopies.

It is commonly accepted that colitis-associated CRC develops along the inflammation- dysplasia-carcinoma-sequence, which is different from the classical adenoma-carcinoma-sequence that is observed in most patients without colitis.[21] Colitis-associated dysplastic lesions are thought to occur multifocally, have a flat architecture, are poorly delineated and are therefore difficult to resect radically during colonoscopy. This type of lesion is mostly referred to as DALM and current surveillance guidelines recommend colectomy if such a lesion is encountered, although there is ongoing debate whether this is the optimal treatment option.[12,22] This is in contrast to the situation with sporadic adenomas that are also detected in patients without colitis, which can generally be visualized without the aid of chromoendoscopy and can more often safely be resected endoscopically.

A meta-analysis focusing on cancer risk after detection of flat dysplasia in longstanding IBD showed an increased risk of developing CRC, while a recent meta-analysis, in whom polypoid lesions were resected, showed a substantially lower cancer risk.[23,24] Apparently, the risk of developing CRC after resection of a dysplastic lesion depends on its endoscopic morphology and therefore probably on its resectability.

Therefore, accurate differentiation between colitis-associated dysplasia and sporadic adenomas appears to be relevant for decision-making in the treatment of dysplastic lesions. As there are no distinctive pathological markers available, differentiation is mainly based on the endoscopic image. Endoscopic differentiation between dysplastic and non-dysplastic lesions in patients with colitis is challenging however, as the mucosal and vascular irregularities caused

by quiescent or active inflammation can mimic the changes observed in dysplastic lesions. A recent study by Allende and colleagues looked at the correlation between endoscopic diagnosis and pathology and showed a poor correlation. In over half of the cases dysplasia was seen histologically in which the endoscopic diagnosis was negative.[26] These results emphasize the difficulties in making an endoscopic diagnosis of lesions found during IBD surveillance colonoscopy.[25]

It has been suggested that the application of dye during colonoscopy could increase differentiation of detected lesions by highlighting its surface pattern.[15] The accuracy of the overall differentiation described in our study (overall 62%, expert 68% and non-expert 58%) was similar to the accuracy observed in the study from Farraye where standard white light endoscopy was used (overall 65%, expert 75% and non-expert 56%).[26] Although comparing the results of two image-based studies is not the optimal study approach, this suggests that the additional value of pCE in differentiating lesions encountered during IBD surveillance is smaller than previously perceived. On the other hand, pCE might delineate dysplastic mucosa more clearly, thereby increasing the chance of complete endoscopic removal.

It should be recognized that our study, focusing on endoscopic diagnosis of lesions found during chromoendoscopic IBD surveillance colonoscopies, has limitations. Every image-based inter-observer study is subject to selection bias. All 30 cases contained only one still image, which was manually selected from the endoscopic databases if it represented a characteristic lesion, if the quality of the picture was sufficient and if histology was available. In daily practice, pictures and biopsies are mostly taken in lesions of which the endoscopist is uncertain about the diagnosis. Another limitation is that still images were used in contrast to videos. The latter would have provided the participants with the opportunity to examine the mucosa from different angles and in perspective to the colon. Because histological outcome and endoscopic management was given after each case, this may have affected the assessments of the subsequent cases by creating a short learning curve. We tried to correct for this by presenting the lesions in random order and adding a confidence level to the diagnosis. Obviously, experts have far more experience in performing surveillance using pCE and are more convinced about their diagnosis than non-experts, resulting in significantly higher confidence levels of the endoscopic diagnosis. We showed that accuracy was positively correlated with the confidence level of the endoscopists. Lastly, we have included mainly lesions containing LGD and no HGD lesions have been included, potentially biasing our results. On the other hand, lesions containing HGD in the setting of IBD are rare, and therefore we assume that the present selection of lesions reliably reflects daily practice.

Our study emphasizes the difficulty for both expert and non-expert endoscopists to differentiate dysplastic from non-dysplastic lesions in IBD patients, despite the improved endoscopic

techniques. Therefore we recommend to remove lesions entirely in case of doubt or, if this appears to be impossible, to take biopsies for histopathological assessment. Consequently, the management of dysplastic lesions should mainly be based on its resectability and not on its endoscopic characteristics, as there is no uniformity in the endoscopic classification.
In conclusion, endoscopists, both experts and non-experts, cannot reliably differentiate between dysplastic and non-dysplastic lesions encountered during surveillance in IBD patients. This emphasizes the value of pathological assessment of all potential dysplastic lesions that are encountered during colonoscopy-surveillance in IBD.

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Chapter 7

Implementing chromoendoscopy for surveillance in inflammatory bowel disease patients does not increase dysplasia detection rate compared to conventional random biopsies: a multicenter case-control study

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Submitted to American Journal of Gastroenterology

ABSTRACT

Introduction

Randomized trials demonstrated that chromoendoscopy is superior to white light endoscopy with random biopsy sampling (WLE) for the detection of dysplasia in patients with inflammatory bowel disease (IBD). Whether implementing chromoendoscopy can increase the detection of dysplasia in clinical practice is unknown.

Methods

Patients with ulcerative colitis (UC) and Crohn's disease (CD) undergoing colonoscopic surveillance between January 2000 and November 2013 in three referral centers were identified using the patients'medical records. In recent years, the use of high-definition chromoendoscopy was adopted in all three centers using segmental pancolonic spraying of 0.1% methylene blue or 0.3% indigo carmine (Chromoendoscopy group). Previously, surveillance was performed employing WLE with random biopsies every 10 cm (WLE group). The percentage of colonoscopies with dysplasia was compared between both groups.

Results

A total of 440 colonoscopies in 401 patients were performed using chromoendoscopy and 1802 colonoscopies in 772 patients using WLE. Except for a higher number of CD patients with extensive disease and more patients with a first degree relative with CRC in the chromoendoscopy group, the known risk factors for IBD-associated CRC were comparable between both groups. Dysplasia was detected during 48 surveillance procedures (11%) in the chromoendoscopy group, compared to 189 procedures (10%) in the WLE group (p=0.80). Targeted biopsies yielded 59 dysplastic lesions in the chromoendoscopy group, which was comparable to the 211 dysplastic lesions detected in the WLE group (p=0.30).

Conclusion

Despite compelling evidence from randomized trials, implementation of chromoendoscopy for IBD surveillance did not increase dysplasia detection compared to WLE with targeted and random biopsies.

INTRODUCTION

Patients with longstanding ulcerative colitis (UC) and Crohn's disease (CD) with colonic involvement have an increased risk of developing colorectal cancer (CRC).[1, 2] Endoscopic surveillance aimed at the detection and treatment of dysplasia and CRC at an early stage is advocated to mitigate this risk, although solid evidence that this strategy is effective is lacking.[3] The detection of neoplasia is challenging, as lesions containing neoplasia are often flat or may not be endoscopically visible at all. Therefore, until recently, surveillance guidelines recommended taking multiple random biopsies throughout the entire colon although 40 to 50 biopsies are needed to achieve an acceptable accuracy for detecting neoplasia.[4, 5] In addition, recently published studies show that almost all neoplastic lesions can be identified endoscopically nowadays, casting further doubt on the practice of taking multiple random biopsies for surveillance purposes.[6-8]

Several randomized trials reported that chromoendoscopy using indigo carmine or methylene blue can increase the neoplasia detection rate substantially compared to white light endoscopy with random biopsy sampling (WLE).[9-12] These findings have prompted the BSG and AGA to advocate chromoendoscopy as the method of choice for CRC surveillance in their updated guidelines.[13, 14] Whether the broad implementation of chromoendoscopy in clinical practice indeed increases the neoplasia detection rate compared to WLE is currently unknown. The aim of the current study was therefore to compare the neoplasia detection rate of colonoscopies performed using chromoendoscopy with procedures performed with WLE.

METHODS

Patients

Patients with a diagnosis of CD or UC were identified in three referral centers using the Diagnosis Treatment Combinations (DTCs) for IBD. DTC's are based on the International Classification of Disease, 9th Revision and can be considered the Dutch version of the Diagnosis Related Groups (DRGs) that are used in other countries.

The medical records and endoscopy reports were reviewed to establish whether patients had a valid indication for CRC surveillance, i.e. patients with a disease duration ≥ eight years and cumulative colonic involvement of at least left sided colitis (UC and IBD-U patients) or more than 30% of the colonic mucosa (CD patients). In addition, patients with colitis and primary sclerosing cholangitis (PSC) were eligible as soon as the combination of these diagnoses was established. The medical records were also reviewed to obtain the patients' demographics, date of IBD diagnosis, type of IBD, disease extent prior to the start of surveillance and family history of CRC.

Details about the family history of CRC were obtained from a questionnaire as part of an observational cohort study for the subgroup of patients that underwent chromoendoscopy.

Surveillance colonoscopies

All surveillance colonoscopies performed between January 2000 and November 2013 in patients with a valid indication for surveillance were collected. Colonoscopies were classified as a surveillance procedure when this was explicitly stated as the indication for the colonoscopy and when either random biopsies were taken or chromoendoscopy was performed.
At the start of the study period, the three centers performed surveillance employing WLE with targeted biopsies of suspicious lesions in combination with four quadrant random biopsies every 10 cm throughout the entire colon, in accordance with international guidelines (WLE group).[4,5] In recent years, all centers adopted pancolonic chromoendoscopy with spraying of either 0.3% indigo carmine or 0.1% methylene blue and targeted biopsies of suspicious lesions as the preferred surveillance method (chromoendoscopy group). Each surveillance colonoscopy was included in the chromoendoscopy or the WLE group based on the method used as described in the endoscopy report. Since this was a retrospective analysis over a 13-year period, multiple surveillance colonoscopies per patient were performed and therefore patients could be included in the WLE group as well as in the chromoendoscopy group. All surveillance procedures during the study period were performed by or under close supervision of experienced gastroenterologists. Procedures in which bowel preparation was deemed inadequate by the endoscopist or in which the cecum was not reached were excluded. Surveillance procedures that were aborted due to the presence of severe inflammation were excluded as well.

Neoplasia

The size, location and endoscopic description of all lesions suspected of containing neoplasia which were biopsied or removed endoscopically or surgically were recorded. Lesions were classified as non-neoplastic, low-grade dysplasia (LGD), high-grade dysplasia (HGD) or CRC based on the pathology report. Discrete solitary sessile or pedunculated polyps resembling sporadic adenomas and showing adenomatous tissue on histology were classified as adenomas whereas all other endoscopic abnormalities (i.e. plaque-like lesions, irregular masses) containing neoplasia were classified as non-adenoma-like masses. In the WLE group, both the total number of random biopsies and the presence of neoplasia in these biopsies were recorded.

Comparison between the chromoendoscopy and WLE procedures

The percentage of colonoscopies with neoplasia (neoplasia yield) was compared between all procedures performed with chromoendoscopy and those performed with WLE. In case of

the WLE group, this comprised neoplasia in targeted as well as in random biopsy samples. The total number of endoscopically visible lesions containing neoplasia was also compared between both groups.
In the subgroup of patients in whom a WLE procedure was followed by a chromoendoscopy procedure during the study period, a direct comparison of the neoplasia detection rate between both surveillance methods was made within the same patient. Since multiple consecutive surveillance procedures might decrease the neoplasia yield of later colonoscopies, the same comparison was made between all patients in whom two subsequent WLE procedures were performed as a reference.

Statistics

Baseline characteristics of the chromoendoscopy and WLE groups were compared using Pearson's chi-squared analysis for categorical variables and Student's t-test or Mann-Whitney U test for continuous variables, depending on whether data were normally distributed. The percentage of colonoscopies with neoplasia was compared between the chromoendoscopy and WLE groups using Pearson's chi-squared analysis.
The comparison between the neoplasia detection rate of chromoendoscopy and WLE within the same patients was made using the Mc Nemar test. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 20 (Chicago, IL, USA) for Windows.

RESULTS

In total, 937 patients (35% CD, 65% UC or IBD-U) underwent 2242 surveillance colonoscopies during the study period. Chromoendoscopy was performed in 440 procedures in 401 patients whereas WLE was performed in 1802 procedures in 772 patients (Figure 1). Baseline characteristics are shown in Table 1. A subgroup of 236 patients underwent WLE as well as chromoendoscopy during the study period. The number of CD patients with extensive colitis and the number of patients with a first-degree relative diagnosed with CRC were significantly higher in the chromoendoscopy group (66% vs 51% and 16% vs 4%, both p<0.01, table 1). The difference in the percentage of patients with a positive family history of CRC could be explained by missing data in the WLE group, as the difference was no longer significant after excluding patients with missing data (22% vs 24%, p=0.72). Furthermore, the mean interval between the prior 'pre-study' surveillance colonoscopy and the chromoendoscopy procedure was significantly longer compared to the surveillance interval in the WLE group (2.8 compared to 2.4 years, p=0.01).

There were no significant differences between the chromoendoscopy and WLE group with regard to other established risk factors for IBD-associated CRC (Table 1).

Figure 1: flow chart showing the patients included in each group

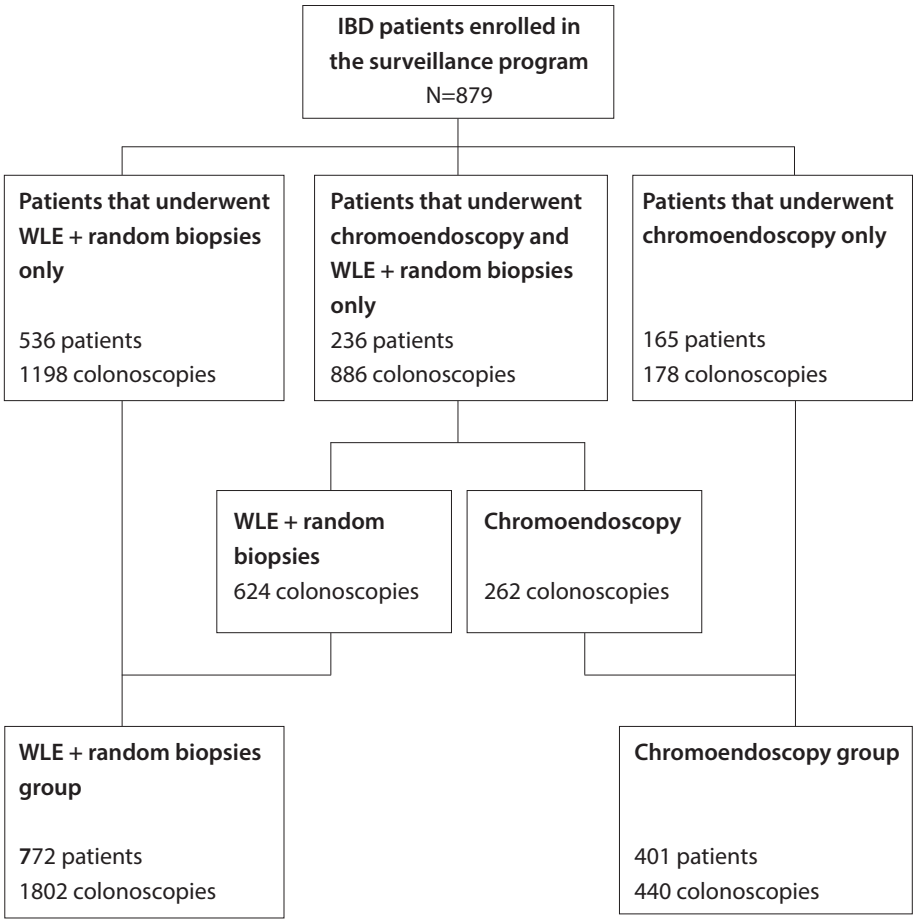


Table 1: baseline characteristics of the patients that underwent chromoendoscopy and WLE + random biopsies

	Chromoendoscopy N (%)	**WLE + random biopsies N (%)**	**p-value**
Number of patients	401 (100)	772 (100)	NA
Male gender	203 (51)	399 (52)	0.71
Age (mean ± SD)	49 ± 12	49 ± 13	0.80
Age at IBD diagnosis, years (mean ± SD)	29 ± 13	28 ± 16	0.76
Duration of IBD, years (mean ± SD)	20 ± 12	20 ± 15	0.95
IBD diagnosis			
Ulcerative colitis	239 (60)	464 (60)	0.49
Distal splenic flexure	85 (36)	175 (38)	0.77
Proximal splenic flexure	141 (56)	270 (58)	
Unknown	14 (6)	20 (4)	
Crohn's colitis	148 (37)	269 (35)	0.49
Segmental colitis <50%	47 (32)	114 (42)	**<0.01**
Segmental colitis >50%	98 (66)	137 (51)	
Unknown	3 (2)	18 (7)	
Indeterminate colitis	13 (3)	35 (5)	0.49
Segmental colitis <50%	3 (23)	18 (51)	0.15
Segmental colitis >50%	10 (77)	16 (46)	
Unknown	0 (0)	1 (3)	
Surveillance interval, years (mean ± SD)	2.8 ± 1.7	2.4 ± 1.3	**0.01**
Concomitant diagnosis of PSC	40 (10)	68 (9)	0.51
First degree relative with CRC diagnosis			**<0.01**
Yes	63 (16)	30 (4)	
No	223 (55)	97 (13)	
Unknown	115 (29)	645 (83)	
Presence of post-inflammatory polyps	80 (20)	166 (22)	0.36

Neoplasia detection rate

In total, neoplasia was detected in 237 surveillance colonoscopies (11%) during the study period. LGD was detected in 227 procedures (10%), HGD in six procedures (0.3%) and CRC in four procedures (0.2%). The overall neoplasia detection rate was comparable between the three centers (11% vs 9% vs 11% respectively, p=0.45). When surveillance colonoscopies were reviewed in chronological order, the neoplasia detection rate remained stable over time (figure 2). The number of surveillance procedures that each patient underwent had no effect on the neoplasia detection rate. In 9% of cases, dysplasia was detected during the first procedure, which was comparable to the detection rate in the second (9%), third (15%) and fourth (10%) surveillance colonoscopy among the patients that underwent multiple consecutive surveillance procedures (p=0.10, Figure 3).

Figure 2: Neoplasia detection rate over time when each surveillance colonoscopy is placed in chronological order

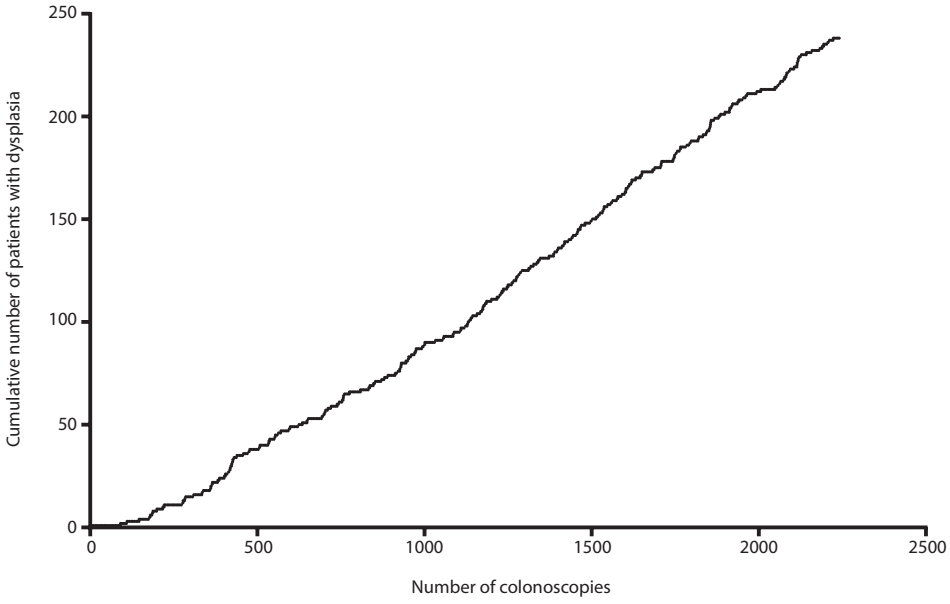
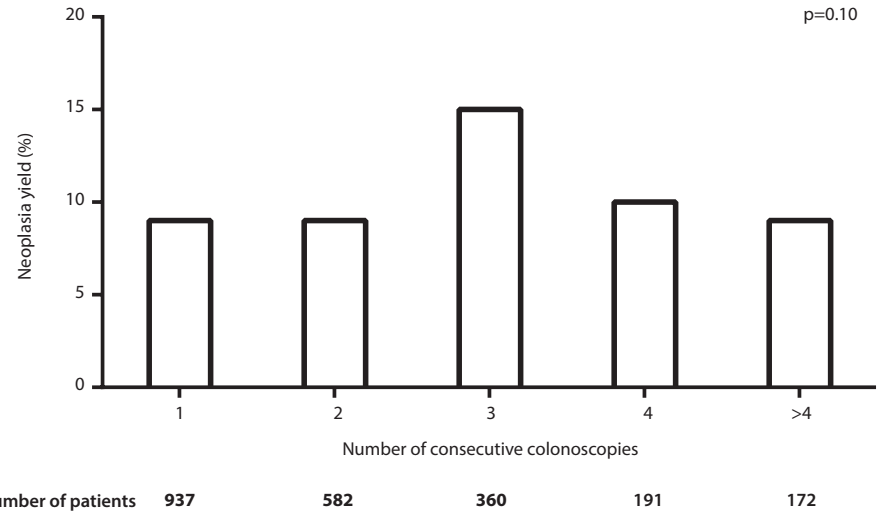


Figure 3: neoplasia detection rate among patients that underwent multiple surveillance colonoscopies during the study period



Neoplasia detection per surveillance method

Neoplasia was detected in 48 out of 440 surveillance procedures performed with chromoendoscopy (11%) and in 189 of the 1802 procedures performed with WLE (10%, p=0.80). If the 236 patients that underwent both chromoendoscopy and WLE were excluded from the analysis, the neoplasia detection rate remained comparable between the chromoendoscopy group (10%) and the WLE group (11%, p=0.65). The similarity in neoplasia detection between chromoendoscopy and WLE procedures was observed in all three centers (11% vs 11%, p=0.91, 8% vs 9%, p=0.65, 12% vs 10% p=0.60 respectively).

Comparison between chromoendoscopy and WLE procedures within the same patients

Of the 236 patients that underwent both chromoendoscopy and WLE during the study period, five were excluded because they underwent WLE after chromoendoscopy. Neoplasia was detected in 31 (13%) of the WLE procedures and in 30 (13%) of the chromoendoscopy procedures (p=1.0). As a reference, 661 paired consecutive WLE procedures were available of which the neoplasia detection rate was 8% in the first procedure compared to 11% in the following procedure (p=0.05).

Neoplasia characteristics per surveillance method

In the chromoendoscopy group, targeted biopsies were sampled from 546 lesions (mean 1.2 per procedure), which was significantly higher than the 1069 lesions (mean 0.6 per procedure) biopsied in the WLE group (p<0.01, table 2). The targeted biopsies in the chromoendoscopy group identified neoplasia in 48 procedures (11%), which was similar to the 158 procedures (9%) with neoplasia identified with targeted biopsies in the WLE group (p=0.19). The number of lesions with neoplasia was also comparable between the chromoendoscopy and WLE group (p=0.30, table 2).

The 51,602 random biopsies sampled in the WLE group yielded an additional 31 procedures (1%) in which neoplasia was detected. In all cases, this was found to be LGD.

Table 2: Neoplasia characteristics of the lesions detected in the chromoendoscopy and the WLE + random biopsy group

	Chromoendoscopy	**WLE + random biopsies**	**p-value**
Number of colonoscopies	440	1802	NA
Total number of lesions detected (mean ± SD)	546 (1.2 ± 1.5)	1069 (0.6 ± 1.2)	<0.01
Total number of random biopsies (mean ± SD)	NA	51602 (29 ± 9)	NA
Procedures with neoplasia (%)	48 (11)	189 (10)	0.80
Targeted biopsies	48 (11)	158 (9)	0.19
Random biopsies	NA	31 (1)	NA
Lesions containing neoplasia (mean ± SD)	59 (0.13 ± 0.47)	211 (0.12 ± 0.44)	0.30
Adenoma	53	185	0.15
Non-adenomatous lesion	6	16	0.24
HGD	0	6	0.23
CRC	0	4	0.32
Size of the lesions with neoplasia median (range)	4 (1 - 30)	3 (1 - 50)	0.41

DISCUSSION

This large retrospective study showed that the implementation of chromoendoscopy with targeted biopsies as the preferred surveillance method did not result in an increased neoplasia detection rate as compared to WLE with random biopsies.

This finding is in contrast with several controlled trials, which showed a substantial increase in neoplasia detection using chromoendoscopy compared to WLE.[9-12] Kiesslich et al. reported an impressive increase of dysplasia yield using chromoendoscopy as compared to WLE from 6% to 15% (combined data from 2 randomized controlled trials).[10, 12] Two other studies employing a back-to-back study design reported that among 202 procedures, the second withdrawal using chromoendoscopy increased the neoplasia detection rate from 7% to 13%.[9, 11] Since dye-spraying cannot be undone, the two back-to-back studies could not perform WLE after chromoendoscopy which may have overestimated the additional neoplasia yield of chromoendoscopy in these particular studies. Studies in non-IBD patients using a back-to-back design have shown that the second colonoscopy, even when using standard WLE colonoscopy in both cases, increases the adenoma detection rate substantially.[15] It is therefore conceivable that the same phenomenon could have resulted in higher yields in the second procedure in these colitis studies as well. Furthermore, the endoscopist cannot be blinded for the surveillance method. It could be that the expectation that chromoendoscopy performs better introduced a bias, despite randomization.

The combined neoplasia detection rate using WLE from the randomized trials and back-to-back studies was substantially lower than the rate in our study (7% vs. 10%), while the rate for chromoendoscopy was found to be higher compared to our results (14% vs. 11%). These divergent outcomes may be due to several factors. First, the higher neoplasia detection rates in previous trials might be due to the fact that expert endoscopists with extensive experience performed the chromoendoscopy procedures. Obviously, this would not explain the lower neoplasia yield of the WLE arm in the RCT's. Since all three centers in our study started using chromoendoscopy during the study period, the initial learning curve might have lowered the total neoplasia detection rate. Of note, the procedures in our cohort were performed or supervised by endoscopists with extensive experience in surveillance colonoscopies for colitis, providing a near optimal setting for surveillance. Second, the absence of a strict protocol for surveillance colonoscopies in the three participating centers may have affected the quality of the procedures. Since bowel preparation and the absence of inflammation are paramount for the detection of subtle dysplastic lesions, especially when using chromoendoscopy [16], the lack of a standardized approach might have resulted in the inclusion of low-quality procedures and missed lesions in our study. Again, this would not explain the relative low neoplasia yield in the

RCT's and high neoplasia yield in our series in the WLE group. Third, since the study period spanned more than a decade, different types of endoscopes were used over time, which could have influenced neoplasia detection rates. Although data on the type and characteristics of the endoscopes used were not collected, the bulk of the chromoendoscopy procedures was performed in the last few years of the study period and therefore these procedures benefitted from better endoscopes with a higher resolution. This could have led to a higher neoplasia detection rate in our chromoendoscopy group and a gradual overall increase of neoplasia yield in both the chromoendoscopy and WLE groups over time. However, the data from the current study do not support this assumption (Figure 2).

The retrospective nature of our study might have introduced several forms of bias. Since our study covers a period of more than10 years, it is conceivable that a change of the incidence of neoplasia has influenced our results. Several epidemiological studies have shown that the risk of CRC is decreasing in IBD patients, possibly due to improved treatment and/or better implementation of endoscopic surveillance.[17, 18] By plotting each surveillance colonoscopy in chronological order, we found that the neoplasia detection rate remained remarkably stable throughout the study period, suggesting this did not influence our results (Figure 2). Theoretically however, the decreased incidence of neoplasia in IBD over time could have been balanced out by the increased neoplasia yield using chromoendoscopy and/or better endoscopes.

The fact that this study was retrospective and not randomized might well have resulted in a difference in patient characteristics between the chromoendoscopy and WLE group. We indeed found that the percentage of CD patients with extensive colitis and patients with a positive family history for CRC was higher in the chromoendoscopy group. Since these are both risk factors for CRC, these differences should have caused a higher rather than a lower dysplasia detection rate. The interval between the last 'pre-study' surveillance colonoscopy and the procedures performed with chromoendoscopy was also significantly longer compared to the surveillance intervals in the WLE group. Again, as a longer surveillance interval theoretically results in more time for neoplasia to develop, this bias would only increase the neoplasia detection rate. Of course, some level of residual confounding is undoubtedly present.

Despite the fact that there were no significant differences in both the number of lesions or procedures with neoplasia between the chromoendoscopy and WLE groups, it is conceivable that chromoendoscopy aids in the detection of more subtle, small and flat dysplastic lesions.[10] The fact that the size of the dysplastic lesions was comparable in both groups does not support this notion however.

Another issue that cannot be addressed by the current study design is the clinical relevance of the neoplastic lesions detected by both surveillance techniques. The ultimate goal of surveillance is to detect and treat dysplastic lesions that would otherwise progress to CRC and

thereby decrease CRC-related mortality. Especially for LGD detected in random biopsies, there has been much debate on whether this finding is clinically relevant. Although some studies showed that between 37% and 53% of patients with confirmed LGD detected in random biopsies progress to HGD or CRC during follow-up,[19, 20] others have reported that the incidence of dysplasia in random biopsies is extremely low and that this usually has no consequences for the follow-up strategy.[6] The same holds true for the additional neoplasia detected with chromoendoscopy compared to conventional WLE. It has been suggested that these lesions are often small and flat, but there are no follow-up studies on the progression rates to advanced neoplasia of these lesions.

If future prospective studies will confirm our results, the question arises what the role of chromoendoscopy should be in the setting of CRC surveillance in IBD patients. Chromoendoscopy was initially commended for its superior neoplasia detection, but it may also be less costly than WLE plus random biopsies as well, which in itself could be an incentive to prefer chromoendoscopy.[21] Furthermore, chromoendoscopy might aid in assessing the pit-pattern of lesions and thereby aiding the differentiation between neoplastic and non-neoplastic lesions. On the other hand, it is conceivable that the targeted biopsies guided by the present, state-of-the-art, high definition colonoscopes can provide the same neoplasia detection rates as chromoendoscopy without taking additional random biopsies, rendering chromoendoscopy as a flagging tool redundant in the near future.

In conclusion, we did not find an increase in neoplasia detection after the implementation of chromoendoscopy as compared to the conventional WLE plus random biopsies protocol. Although more studies are needed to confirm this, these results cast doubt on the standard use of chromoendoscopy as the preferred surveillance tool in IBD.

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Part II:

Biomarkers for inflammation

Chapter 8

Fecal hemoglobin and calprotectin are equally effective in identifying inflammatory bowel disease patients with active endoscopic inflammation

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Published in Inflammatory Bowel Diseases

ABSTRACT

Background

Fecal calprotectin can be used as a non-invasive tool to assess inflammation in patients with inflammatory bowel disease (IBD). However, the diagnostic accuracy of calprotectin is modest and therefore additional markers are needed. We compared the efficacy of fecal hemoglobin and calprotectin as markers for endoscopic inflammation in IBD patients.

Methods

Consecutive patients with Crohn's disease (CD) or ulcerative colitis (UC) scheduled for surveillance colonoscopy collected a stool sample prior to bowel preparation.
Experienced endoscopists assessed the presence of inflammation in each colonic segment. Fecal calprotectin and hemoglobin were analyzed with an enzyme-linked immunosorbent assay. ROC statistics were used to determine cut-off values for calprotectin and hemoglobin.

Results

A total of 176 surveillance colonoscopies were performed in 164 patients, of which 83 patients had CD, 74 had UC and seven IBD-unclassified. Median (interquartile range (IQR)) calprotectin and hemoglobin concentrations were 137 mg/kg (IQR 33 – 494) and 0.51 µg/g (IQR 0.18 – 8.50) respectively. For calprotectin, a cut-off value of 140 mg/kg predicted endoscopic inflammation with 86% sensitivity, 72% specificity, 64% positive predictive value (PPV), 90% negative predictive value (NPV) and an area under the curve (AUC) of 0.87. For hemoglobin, a cut-off value of 1.51 µg/g indicated endoscopic inflammation with 74% sensitivity 84% specificity, 72% PPV, 84% NPV and an AUC of 0.81. Combining both tests did not increase the predictive accuracy substantially compared to calprotectin or hemoglobin alone (AUC 0.88).

Conclusion

Fecal hemoglobin can identify IBD patients with active inflammation with a predictive accuracy similar to calprotectin.

INTRODUCTION

Patients with ulcerative colitis (UC) and Crohn's disease (CD) suffer from recurrent episodes of inflammation of the gastrointestinal tract. Since clinical symptoms are known to correlate poorly with objective measures of disease activity in both UC and CD patients, tailoring treatment to the presence of inflammation is challenging.[1-3] The current gold standard for the assessment of ileocolonic inflammation is colonoscopy, which is expensive, burdensome for patients and associated with a risk of complications.[4] Less invasive markers of mucosal inflammation, such as fecal calprotectin, seem to be promising alternatives. Several studies have shown that calprotectin concentrations correlate reasonably well with endoscopic disease activity in both CD and UC.[1, 5, 6] Currently, calprotectin is widely used as a marker for mucosal inflammation in clinical practice, but its diagnostic accuracy is imperfect and therefore clincial decision making cannot be based solely on this parameter.

Determination of fecal hemoglobin is used as a screening method for colorectal cancer in the general population.[7] Since occult intestinal blood loss is an important symptom in patients with inflammatory bowel disease (IBD), fecal hemoglobin could potentially be used as a marker for mucosal inflammation in these patients as well. One previous study in patients with UC indeed showed that the concentration of fecal hemoglobin correlated well with the endoscopic severity of inflammation.[8] Whether fecal hemoglobin is inferior or superior to calprotectin in identifying patients with mucosal inflammation is currently unknown. Therefore, the aim of the present study was to compare the performance of fecal hemoglobin and calprotectin as markers for the presence of endoscopic inflammation in patients with CD and UC.

METHODS

Patients

Consecutive patients with Crohn's colitis,ulcerative colitis or IBD-unclassified (IBD-U) visiting the outpatient clinic and who were scheduled for a surveillance colonoscopy were asked to participate in the current study. Therefore, patients enrolled in the study had a history of at least left sided colitis (UC and IBD-U patients) or colitis involving at least 30% of the colonic mucosa (CD patients). Participating patients collected a sample of feces within two days prior to the scheduled colonoscopy and before the start of bowel cleansing. Fecal samples were stored at room temperature by the patients until colonoscopy. All patients completed a questionnaire to collect data for the simple clinical colitis activity index (SCCAI, UC and IBD-U patients) or the Harvey Bradshaw index without the parameter abdominal mass (CD patients).[9, 10] Bowel preparation was performed with 4 litres of polyethylene glycol according to the

standard protocol in our center. Three senior gastroenterologists performed the surveillance colonoscopies and scored the endoscopic disease severity and extent of inflammation in all segments of the colon (caecum and ascending colon combined, transverse colon, descending colon and rectosigmoid). Disease severity in each segment was scored as no, mild (erythema, decreased vascular pattern, mild friability), moderate (marked erythema, absent vascular pattern) and severe (spontaneous bleeding, ulceration) inflammation in accordance with the Mayo endoscopic score.[11] This tool was used in UC, IBD-U and CD patients, since all patients had colonic disease involvement. The number of inflamed colonic segments was multiplied by the endoscopic severity score to obtain the total inflammation score ranging from 0 (no inflammation) to 12 (severe pancolitis).
Patients in whom the caecum could not be reached or with insufficient bowel preparation to assess endoscopic disease severity were excluded from further analysis. The gastroenterologists were blinded for fecal hemoglobin and calprotectin levels when performing the colonoscopies.

Fecal hemoglobin and calprotectin analysis

After the fecal samples were received from the patients they were stored at -80 °C until shipment to the manufacturer for hemoglobin and calprotectin analysis. Both hemoglobin and calprotectin were measured using a quantitative enzyme linked immuno assay according to the manufacturer's instructions (Ridascreen Hemoglobin and Ridascreen Calprotectin, provided by R-Biopharm, Darmstadt, Germany). The measurement range is between 19.5 and 800 mg/kg for calprotectin and between 0.42 and 50 µg/g for fecal hemoglobin at a final dilution of 1:2500. Samples with calprotectin or hemoglobin levels at the upper limit were diluted further and measured again to obtain a quantitative value. Calprotectin levels were also analysed with the commonly available Buhlmann assay (Buhlmann laboratories AG, Basel Switzerland) which showed an excellent correlation with the Ridascreen results (correlation coefficient r=0.93). Samples were analyzed without knowledge of the colonoscopy results.

Statistical analysis

Baseline characteristics were analyzed using standard descriptive statistics. Spearman rank correlation test was used to assess the correlation between levels of fecal calprotectin and fecal hemoglobin and the total endoscopic inflammation score in UC and CD patients. Receiver operator characteristic (ROC) curves were constructed to calculate the accuracy of fecal hemoglobin and calprotectin in discriminating between patients with mucosal healing and active endoscopic inflammation and to identify appropriate cut-off values for both markers. The method described by Hanley and McNeil was used to compare the area under the curve (AUC)

of the ROC curves of fecal hemoglobin and calprotectin and to assess whether the ROC curves of fecal hemoglobin and calprotectin were significantly different for UC and CD patients.[12] A two-sided p-value <0.05 was considered statistically significant. All analyses were performed using SPSS version 20 (Chicago, IL, USA) for Windows.

Ethical considerations

This study was carried out with the approval of and in accordance with the ethical guidelines of the research review committee of our institution. All patients gave written informed consent.

RESULTS

Patients

A total of 176 surveillance colonoscopies were performed in 164 patients, the recruitment process is summarized in figure 1. Seventy four patients had a diagnosis of ulcerative colitis (45%), 83 Crohn's colitis (51%) and seven IBD-U (4%). Baseline characteristics of patients are shown in table 1.

Figure 1: Flow chart showing the recruitment process of the study population.

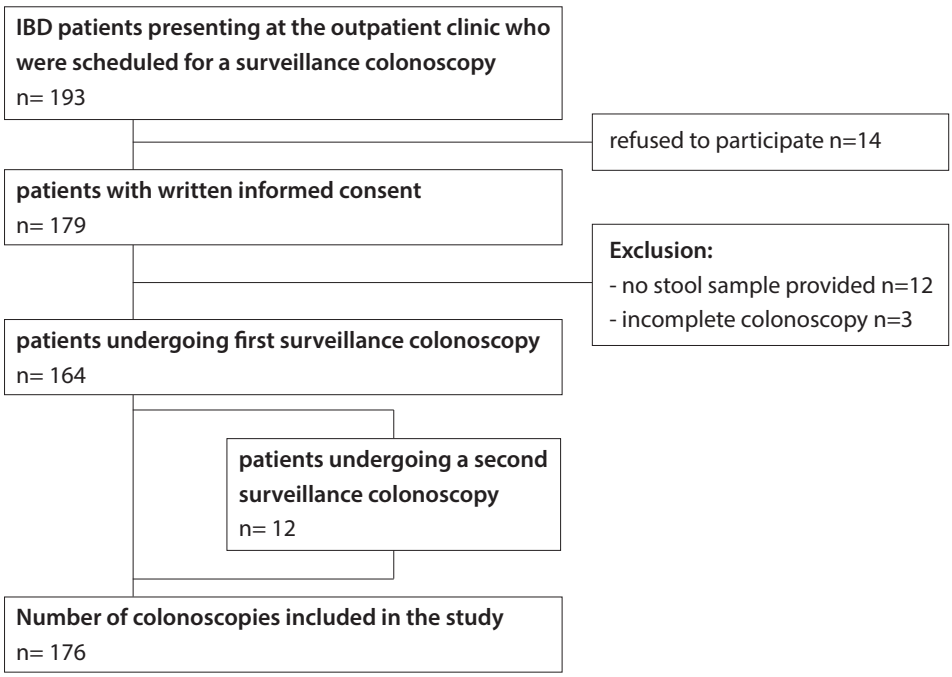


Table 1: Baseline characteristics of IBD patients undergoing surveillance colonoscopy

	N (%)
Number of patients	164 (100)
Male gender	81 (49)
Age, years (median, range)	49 (19 – 72)
Duration of IBD, years (median, range)	19 (1 – 51)
IBD diagnosis	
Ulcerative colitis	74 (45)
Left-sided	25 (34)
Extensive	45 (61)
Unknown	4 (5)
IBD-unclassified	7 (4)
Left Sided	3 (43)
Extensive	4 (57)
Unknown	0 (0)
Crohn's colitis	83 (51)
Segmental colitis <50%	24 (29)
Segmental colitis >50%	58 (70)
Unknown	1 (1)
Age at diagnosis (years, CD patients)	
A1	11 (13)
A2	61 (74)
A3	11 (13)
Disease phenotype (CD patients)	
B1	39 (47)
B1p	9 (11)
B2	20 (24)
B2p	8 (10)
B3	6 (7)
B3p	1 (1)
Disease location (CD patients)	
L1	0 (0)
L2	41 (49)
L3	37 (45)
L2 + L4	2 (2)
L3 + L4	3 (4)
Concomitant diagnosis of PSC	22 (13)
Medication use	
5-ASA	91 (55)
Thiopurines	65 (40)
Methotrexate	5 (3)
Biologicals	33 (20)

IBD, inflammatory bowel disease; PSC, primary sclerosing cholangitis.

Fecal hemoglobin and calprotectin in Crohn's colitis patients

In total, 89 colonoscopies were performed in 83 patients with Crohn's colitis. Fourty patients (48%) had a history of ileocolonic disease and 43 patients (52%) had only colonic disease (table 1). The median Harvey Bradshaw score was 0 (range 0 – 15) and 9 patients (10%) had scores above five.

Endoscopic signs of colonic inflammation were found in 37 patients (42%) of whom 23 patients (26%) had mild inflammation, 8 patients (9%) moderate inflammation and 6 patients (7%) severe inflammation. Multiplying severity and extent of inflammation yielded a median total inflammation score of 0 (range 0 – 10). The Harvey Bradshaw scores correlated poorly with the total inflammation score, although the correlation was statistically significant (r=0.22 p=0.03).

The median fecal hemoglobin and calprotectin levels were 212 mg/kg (range 20 – 26336 mg/kg) and 0.78 µg/g (range 0 – 896 µg/g), respectively. Both markers showed a statistically significant correlation with the total endoscopic inflammation score (r=0.44 for fecal hemoglobin and r=0.54 for calprotectin, p<0.01) (figure 2 and 3).

The terminal ileum was inflamed in 12 patients (13%), no inflammation was found in 65 patients (74%) and the terminal ileum could not be intubated in 12 patients (13%). If the terminal ileum was added to the total endoscopic inflammation score as an additional segment, the correlation coefficients of fecal hemoglobin and calprotectin changed only marginally: 0.52 vs 0.54 with the terminal ileum included for calprotectin and 0.43 vs 0.44 with the terminal ileum included for fecal hemoglobin.

Figure 2: Scatterplot showing the correlation between fecal calprotectin levels and the endoscopic inflammation score for patients with Crohn's colitis (left figure) and ulcerative colitis (right figure). The number of inflamed colonic segments was multiplied by the endoscopic severity score to obtain the total inflammation score ranging from 0 (no inflammation) to 12 (severe pancolitis).

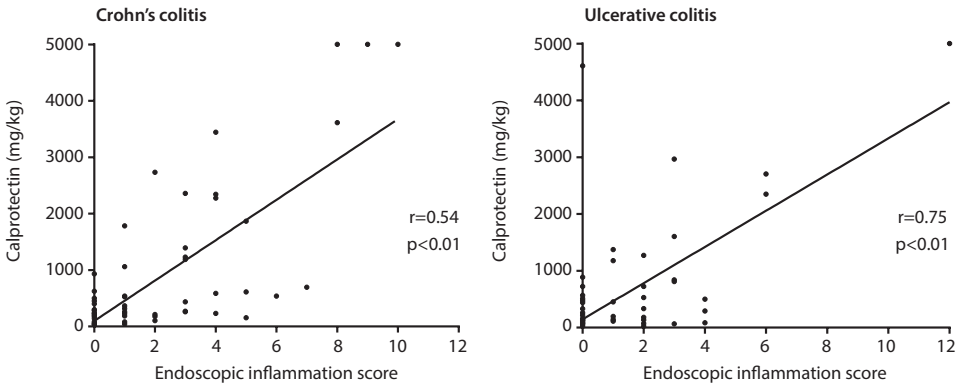
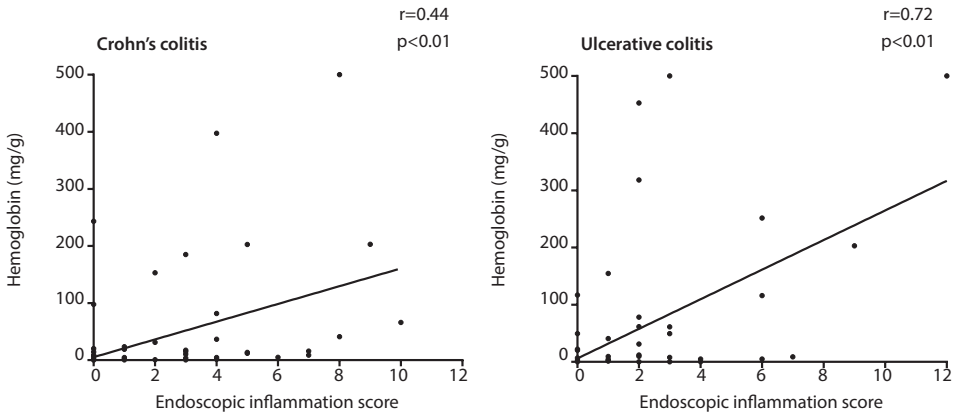


Figure 3: Scatterplot showing the correlation between fecal hemoglobin levels and the endoscopic inflammation score for patients with Crohn's colitis (left figure) and ulcerative colitis (right figure). The number of inflamed colonic segments was multiplied by the endoscopic severity score to obtain the total inflammation score ranging from 0 (no inflammation) to 12 (severe pancolitis).



Fecal hemoglobin and calprotectin in ulcerative colitis patients

Eighty colonoscopies were performed in 74 ulcerative colitis patients. Median SCCAI score was 0 (range 0 – 11) and only 2 patients (3%) had scores above five. No endoscopic inflammation was found in 53 patients (66%), mild inflammation in 16 patients (20%), moderate inflammation in 7 patients (9%) and severe inflammation in 4 patients (5%). Multiplying severity and extent of inflammation resulted in a median total inflammation score of 0 (range 0 – 12). The SCCAI correlated poorly with the total inflammation score, although the correlation was statistically significant (r=0.35 p<0.01).

Median fecal hemoglobin and calprotectin levels were 115 mg/kg (range 20 – 14189 mg/kg) and 0.37 µg/g (range 0 – 3342 µg/g), respectively, which was similar to CD patients (p=0.32 for fecal hemoglobin and p=0.11 for calprotectin). Both markers showed a statistically significant correlation with the total endoscopic inflammation score (r=0.75 for calprotectin and r=0.72 for fecal hemoglobin, p<0.01)(figure 2 and 3).

Comparison between fecal hemoglobin and calprotectin

There was a good correlation between fecal hemoglobin and fecal calprotectin concentrations in both ulcerative colitis and Crohn's colitis patients (r=0.93 and r=0.88 respectively, p<0.01). When CD and UC patients were combined, the ROC curve for fecal hemoglobin showed that a cut-off value of 1.51 µg/g resulted in 74% sensitivity, 84% specificity, a positive predictive value (PPV) of 72% and a negative predictive value (NPV) of 84% in identifying patients with active

endoscopic inflammation with a corresponding AUC of 0.81 (figure 3).
For calprotectin, a cut-off value of 140 mg/kg resulted in 86% sensitivity, 72% specificity, 64% PPV and 90% NPV in identifying CD and UC patients with active endoscopic inflammation with a corresponding area under the curve of 0.87 (figure 4).
Although the predictive accuracy of calprotectin was better than fecal hemoglobin (AUC 0.87 vs 0.81, respectively) this difference was not statistically significant (p=0.06).
The diagnostic accuracy of fecal hemoglobin was better in patients with CD compared to UC patients, but this difference was not statistically significant (AUC 0.82 and 0.77, respectively, p=0.52). Similarly, the diagnostic accuracy of calprotectin was slightly better in patients with CD compared to patients with UC as well (AUC 0.89 vs 0.82 respectively, p=0.26).
When calprotectin and fecal hemoglobin were combined in one ROC curve, the AUC increased only marginally to 0.88 as compared to 0.87 for calprotectin alone and 0.82 for fecal hemoglobin alone.
Furthermore, we assessed whether a strategy of fecal hemoglobin testing after calprotectin testing could improve the accuracy of identifying patients with endoscopic inflammation (figure 5). Applying the predefined cut-off values of 140 mg/kg for calprotectin and 1.51 µg/g for fecal hemoglobin, 87 patients had levels above 140 mg/kg of which 59 patients had fecal hemoglobin levels above 1.51 µg/g as well. Calprotectin levels below 140 mg/kg and fecal hemoglobin levels below 1.51 µg/g were observed in 82 patients. If both markers were elevated above the cut-off values, this indicated endoscopic inflammation with a 88% sensitivity, 85% specificity, a PPV of 78% and a NPV of 91%. When compared to calprotectin testing alone, the PPV increased from 64% to 78% and the specificity from 72% to 85% whereas the NPV and sensitivity increased only marginally from 90% to 91% and 86% to 88%, respectively.

Figure 4: Receiver operator curve for fecal hemoglobin and calprotectin showing the diagnostic accuracy for distinguishing any inflammation from no inflammation.

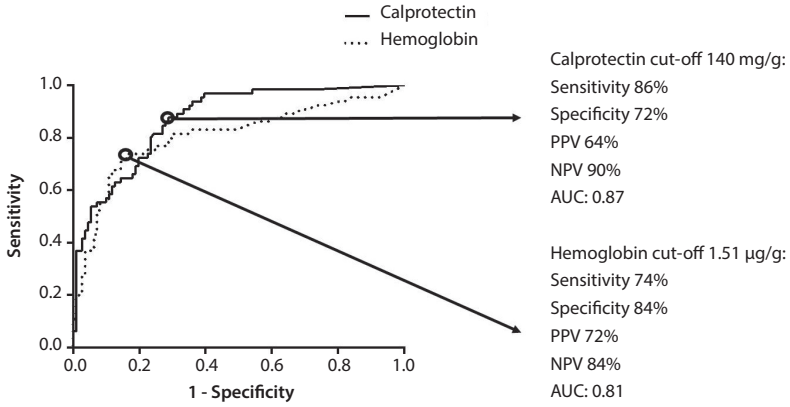
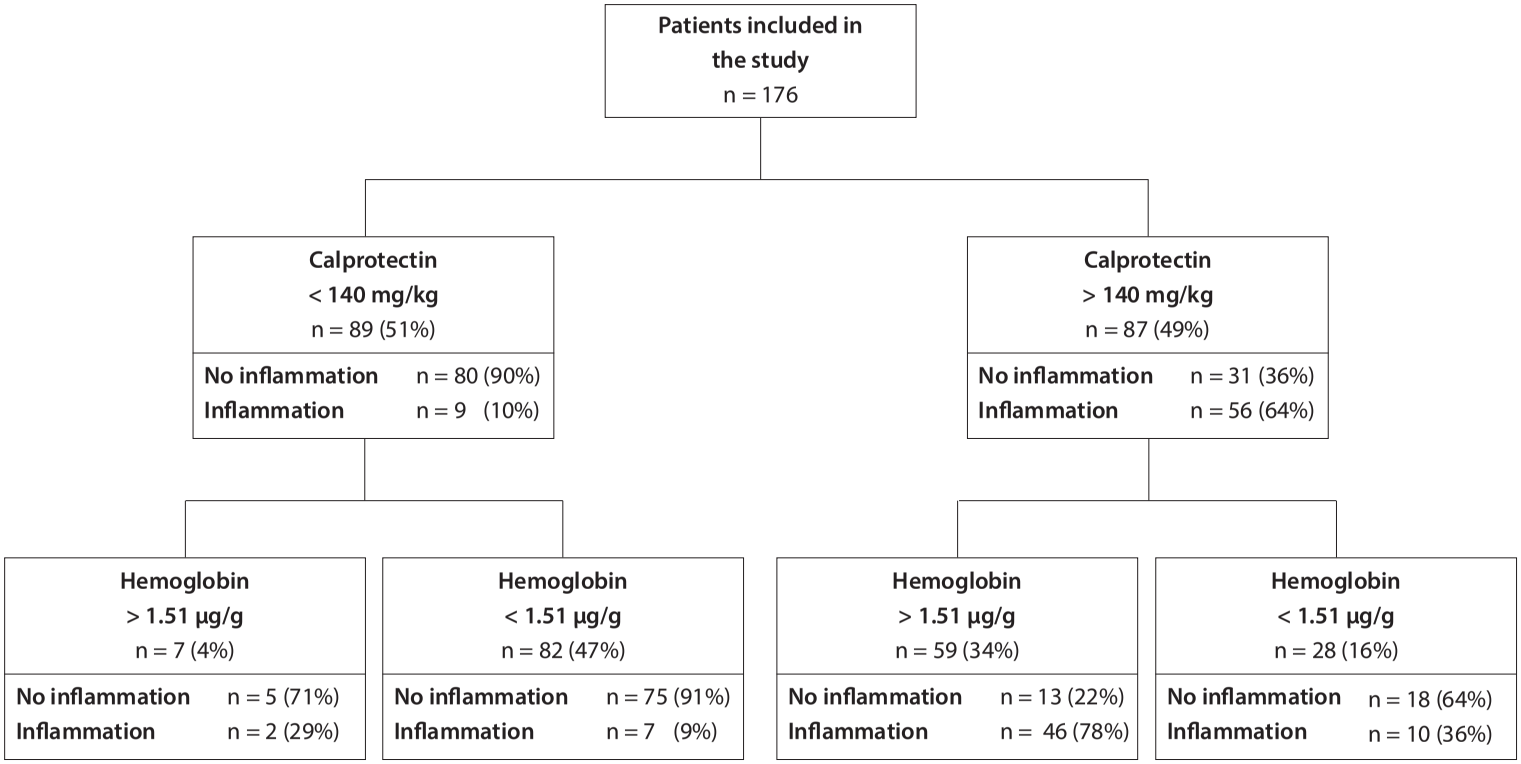


Figure 5: Flow chart showing the number of patients with inflammation using the predefined cut-offs for fecal hemoglobin and calprotectin.



DISCUSSION

This study demonstrates that fecal hemoglobin is a marker for colonic inflammation in patients with Crohn's disease and ulcerative colitis with a comparable diagnostic accuracy to fecal calprotectin.

Several previous studies have confirmed the correlation between endoscopic inflammation and calprotectin levels in both CD and UC. Our correlation coefficients of 0.54 for patients with CD and 0.75 for patients with UC are in line with these previous reported correlation coefficients ranging from 0.42 to 0.79 for CD and from 0.51 to 0.83 for UC. [1, 13-17] The fact that the correlation was better for patients with UC than for patients with CD is also in line with two previous studies that included both UC and CD patients.[11, 14] This might be due to the fact that small bowel mucosal lesions were also present which could result in elevated calprotectin levels without being detected by ileocolonoscopy.

Since we tested fecal hemoglobin in the same samples, we were able to compare the performance of fecal hemoglobin and calprotectin as markers for endoscopic inflammation and to assess whether combining these markers could improve the diagnostic accuracy. One previous study investigated fecal hemoglobin as a marker for inflammation in 152 patients with UC.[8] Fecal hemoglobin was assessed using a quantitative fecal immunochemical test (FIT), which is commonly used in sporadic colorectal cancer screening programs.[18] The authors found that levels above a cut-off of 100 ng/ml indicated endoscopic inflammation with 60% sensitivity and 87% specificity. Although we used an enzyme-linked immuno assay to determine fecal hemoglobin levels resulting in a different cut-off level, our results are similar to these data, confirming that fecal hemoglobin is an accurate marker for colonic inflammation.

Furthermore, we found that fecal hemoglobin levels can identify endoscopic inflammation in patients with Crohn's colitis as well, with 73% sensitivity and 79% specificity. The diagnostic accuracy of fecal hemoglobin in identifying patients with inflammation as reflected by an AUC of 0.82 for CD patients and 0.77 for UC patients, which was similar to the AUC of 0.89 (CD patients) and 0.82 (UC patients) for fecal calprotectin. It seems therefore that fecal hemoglobin is equally effective in distinguishing between patients with and without inflammation as calprotectin. Combining both markers improved the predictive accuracy only marginally as compared to each marker alone (AUC 0.88 versus 0.87 and 0.82).

A specific advantage of fecal calprotectin as a marker for inflammation is the fact that it remains stable at room temperature for several days.[19] Regarding hemoglobin in stool samples it is known that some degradation does occur after stool is exposed to air.[20] For this reason, most fecal hemoglobin tests used for the screening of sporadic colorectal cancer use a stabilizing buffer contained in the sampling tube.[7] In the current study fecal samples without a

buffer were collected by the patients prior to the start of bowel cleansing and kept at room temperature for up to three days. Although this most likely resulted in a decline in fecal hemoglobin concentrations in all samples, the correlation coefficients between calprotectin and fecal hemoglobin and endoscopic inflammation were found to be similar. We therefore believe that although fecal hemoglobin is probably not as stable as calprotectin at room temperature, it seems stable enough to be used as an accurate marker for inflammation in fecal samples kept at room temperature for up to three days.

Mucosal healing is associated with a decrease in hospitalizations, number of surgical resections and incidence of colorectal cancer in both UC and CD patients.[21-24] To assess whether calprotectin or fecal hemoglobin could be used as markers for mucosal healing, we determined appropriate cut-off values for both calprotectin and fecal hemoglobin in differentiating between patients with and without endoscopic inflammation.

Previous studies showed that calprotectin cut-off levels of 200 and 250 mg/kg had a high predictive value for active disease ranging between 78% and 100% .[13, 14] We opted for a slightly lower cut-off value of 140 mg/kg which resulted in a markedly higher negative PV of 90% compared to the values ranging between 48% and 61% found by D'Haens et al. and Sipponen et al. [13, 14] Obviously, the drawback of this higher NPV is a lower PPV of 64%. We feel however that if treatment is aimed at achieving mucosal healing, a lower cut-off level with higher NPV's is justified. Calprotectin levels above the cut-off level indicate the presence of inflammation with reasonable accuracy, although this is probably not enough to start treatment and might warrant endoscopic confirmation.

We also assessed cut-off levels for fecal hemoglobin and found that 1.51 µg/g offers the best compromise with a PPV of 72% and a NPV of 84%. As mentioned before, combining fecal hemoglobin and calprotectin testing did not significantly increase the diagnostic accuracy overall. However, if a patient has a calprotectin level above the cut-off of 140 mg/kg, the probability of finding inflammation is raised from 64% to 76% if the fecal hemoglobin level is above the cut-off level as well. In contrast, patients with calprotectin levels below the cut-off level had no inflammation in 90% of cases, which increased only marginally to 91% if fecal hemoglobin was below the cut-off as well. This suggests that fecal hemoglobin testing could be particularly useful in patients with high calprotectin levels to substantiate the conclusion that inflammation is present.

Our study has several limitations that need to be addressed. Due to the design of the study, we only included IBD patients scheduled for endoscopic surveillance for CRC. Therefore, our cohort consists only of patients with longstanding (>8years) IBD with a disease extending proximally to the splenic flexure in UC patients and involving at least 30% of the colonic surface in CD patients. Therefore our results cannot be extrapolated to IBD patients with only

limited colonic involvement and CD patients with only ileal disease.
Since one previous study reported a lower correlation between calprotectin and isolated ileal disease [15], the exclusion of these patients might have overestimated the correlation coefficients of both calprotectin and fecal hemoglobin in CD patients. The addition of the terminal ileum as an extra segment in the total endoscopic inflammation score in CD patients did not result in a higher correlation with calprotectin and fecal hemoglobin, although these results are biased by the fact that the terminal ileum was not introduced in 13% of CD patients.
Furthermore, it is currently unknown what the influence of different types of medication is on calprotectin levels, and therefore the different maintenance therapies of the patients included in the study might have influenced the results.
Surveillance is usually performed when patients are in clinical remission since dysplasia detection is severely impaired when the mucosa shows signs of inflammation. [25] This could have resulted in an underrepresentation of patients with active disease in the current study. If we look at the endoscopic findings however, 37% of patients had signs of endoscopic inflammation, probably because low grade inflammation does not cause an increase in symptoms.[2, 26]
We used the Mayo endoscopic score to assess the endoscopic severity both in patients with UC as well as in patients with CD.[11] Although this scoring system was developed for patients with UC, we opted to use this scoring system to describe the colonic disease severity of CD patients as well since the classification as mild, moderate and severe is considerably easier than the CDEIS score which is specifically designed for CD patients.[27] Since we focused primarily on the differentiation between patients with any inflammation and patients with mucosal healing, we feel the influence of this different endoscopic classification for CD patients is probably small. Furthermore, the fact that we used the same scoring system for CD and UC patients allowed us to combine these patients in the final analysis.
In conclusion, we found that fecal hemoglobin can be used as a marker for colonic inflammation with a similar diagnostic accuracy to fecal calprotectin in patients with CD and UC. Although the combination of both tests did not show a significant improvement in diagnostic accuracy compared to each marker alone, sequential testing increased the probability of detecting inflammation from 64% to 78% when both markers were elevated compared to calprotectin testing alone.

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Chapter 9

Low fecal calprotectin predicts clinical remission in inflammatory bowel disease patients: a plea for deep remission?

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Accepted for publication in Journal of Crohn's and Colitis

ABSTRACT

Background & aims

Mucosal healing has become the treatment goal in patients with ulcerative colitis (UC) and Crohn's disease (CD). Whether low fecal calprotectin levels and histological healing combined with mucosal healing is associated with a further reduced risk of relapses is unknown.

Methods

Patients with CD, UC or IBD-unclassified (IBD-U) scheduled for surveillance colonoscopy collected a stool sample prior to bowel cleansing. Only patients with mucosal healing (MAYO endoscopic score of 0) were included. Fecal calprotectin was measured using a quantitative enzyme-linked immunosorbent assay (R-Biopharm, Germany). Biopsies were obtained from four colonic segments and histological disease severity was assessed using the Geboes scoring system. Patients were followed until the last outpatient clinic visit or the development of a relapse, which was defined as IBD-related hospitalization, surgery or step-up in IBD medication.

Results

Of the 164 patients undergoing surveillance colonoscopy, 92 patients were excluded due to active inflammation or missing biopsies. Of the remaining 72 patients (20 CD, 52 UC or IBD-U), six patients (8%) relapsed after a median follow-up of 11 months (range 5 - 15 months). Median fecal calprotectin levels at baseline were significantly higher for patients who relapsed compared to patients who maintained remission (284 mg/kg vs. 37 mg/kg. $p<0.01$). Fecal calprotectin above 56 mg/kg predicted relapse during follow-up with 100% sensitivity and 64% specificity. The presence of active histological inflammation (Geboes score >3.1) was not associated with an increased risk of relapse ($p=0.94$).

Conclusion

Low calprotectin levels identify IBD patients who remain in stable remission during follow-up.

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are heterogeneous diseases characterized by inflammation of the gastrointestinal tract and a relapsing disease course. Treatment of inflammatory bowel disease (IBD) is currently aimed at achieving mucosal healing, which is reportedly associated with reduced rates of hospitalization and surgery.[1-3] Assessment of disease activity is challenging however, since clinical symptoms are known to correlate poorly with inflammatory status.[4, 5] Therefore, ileocolonoscopy is the current gold standard for the assessment of intestinal inflammation in IBD patients but this procedure is invasive and costly.
Fecal calprotectin is a promising non-invasive marker as it can easily be determined in stool samples and shows a reasonably good correlation with endoscopic inflammation in both CD and UC.[6-8] Surprisingly, patients may display elevated levels of fecal calprotectin even when endoscopic disease activity is absent. This could be due to disease activity proximal to the terminal ileum in CD patients or other pathology in the upper gastro-intestinal tract such as peptic ulcers or nonsteroidal enteropathy.[9] Alternatively, these elevated calprotectin levels could also be explained by low-grade inflammation only detectable upon histological evaluation. Several studies have shown that histological inflammation can be more extensive and severe than endoscopically appreciated.[10, 11] Parallel to the concept of mucosal healing, patients with endoscopic mucosal healing, histological healing and low fecal calprotectin levels might represent a subgroup of patients in 'deep remission' with a lower risk of relapses.[12] Earlier studies have shown that elevated calprotectin levels can predict a relapse during follow-up in patients in clinical remission with a pooled sensitivity of 78% and a specificity of 73%.[13] However, these studies did not incorporate ileocolonoscopy at baseline to confirm mucosal healing.
The aim of the current study was therefore to assess whether elevated fecal calprotectin levels in IBD patients with complete mucosal healing can be explained by histological inflammation. Secondly, we aimed to assess whether histological inflammation and/or elevated calprotectin are predictive of a relapse in these patients.

METHODS

Patients

Consecutive patients with CD, UC or IBD-U in clinical remission scheduled for a surveillance colonoscopy between July 2011 and January 2013 were asked to participate in the current study. Therefore, patients enrolled in the study had to have a history of at least left sided colitis (UC and IBD-U patients) or colitis involving more than 30% of the mucosa (CD patients).

Patients were asked to collect a stool sample within two days prior to bowel cleansing and store this sample at room temperature.
For patients with UC or IBD-U, endoscopic disease severity was scored as no inflammation, mild (erythema, decreased vascular pattern, mild friability), moderate (marked erythema, absent vascular pattern) or severe inflammation (spontaneous bleeding, ulceration) in accordance with the Mayo endoscopic score. Since all enrolled CD patients had colonic disease, we employed the Mayo score for CD patients as well. Patients with mild, moderate or severe inflammation (Mayo 1,2 or 3) in any segment of the colon were excluded from further analysis. In patients with CD, patients with any sign of ileal inflammation (erythema, aphtous lesions or ulcers) or in whom the terminal ileum could not be intubated were excluded.

Fecal calprotectin analysis

Fecal samples were stored at -80 °C until shipment to the manufacturer for calprotectin analysis. Calprotectin was measured using a quantitative enzyme linked immunoassay according to the manufacturer's instructions (Ridascreen Calprotectin, kindly provided by R-Biopharm, Darmstadt, Germany). The measurement range of the calprotectin is between 19.5 and 800 mg/kg and samples were diluted to obtain calprotectin levels above the upper limit. Similar to other calprotectin essays, levels <50 mg/kg were considered normal. R-Biopharm personnel were blinded for the patients' clinical and histological characteristics.

Scoring of histological disease activity

Two biopsies were obtained from four colonic segments in UC and CD patients (eight biopsies in total, ascending colon, transverse colon, descending colon and rectum), and from the terminal ileum in CD patients for the assessment of histological disease severity. Biopsies were fixed in 10% neutral formalin and sections were stained with hematoxylin and eosin. All slides were reviewed by an expert gastrointestinal pathologist who was blinded for the patient's clinical characteristics and fecal calprotectin levels, using the Geboes scoring system. The Geboes scale comprises 7 different parameters: architectural distortion, density of chronic inflammatory infiltrate, density of eosinophils in the lamina propria, density of neutrophils in the lamina propria, the presence of neutrophils in the epithelium, crypt destruction and erosion or ulceration (supplementary table).[14] The most severe grade of inflammation observed in any of the four colonic segments was considered the maximal Geboes score. In accordance with previous studies, a maximal Geboes score of 3.1 or higher was considered as active histological inflammation.[15]
The score for each Geboes parameter in each of the four colonic segments were combined to calculate the total Geboes score, ranging from 0 to 12 for each parameter. For patients with CD, the score for each parameter from the terminal ileum was counted as an additional segment for both the maximal and total Geboes score.

Supplementary table: Geboes histological scoring system for disease severity.

Grade 0	Structural (architectural change)
0.0	No abnormality
0.1	Mild abnormality
0.2	Mild or moderate diffuse or multifocal abnormalities
0.3	Severe diffuse or multifocal abnormalities
Grade 1	Chronic inflammatory infiltrate
1.0	No increase
1.1	Mild but unequivocal increase
1.2	Moderate increase
1.3	Marked increase
Grade 2	Lamina propria neutrophils and eosinophils
2A. Eosinophils	
2A.0	No increase
2A.1	Mild but unequivocal increase
2A.2	Moderate increase
2A.3	Marked increase
2B. Neutrophils	
2B.0	No increase
2B.1	Mild but unequivocal increase
2B.2	Moderate increase
2B.3	Marked increase
Grade 3	Neutrophils in epithelium
3.0	None
3.1	<5% crypts involved
3.2	<50% crypts involved
3.3	>50% crypts involved
Grade 4	Crypt destruction
4.0	None
4.1	Probable – local excess of neutrophils in part of crypt
4.2	Probable – marked attenuation
4.3	Unequivocal crypt destruction
Grade 5	Erosion or ulceration
5.0	No erosion, ulceration, or granulation tissue
5.1	Recovering epithelium + adjacent inflammation
5.2	Probable erosion – focally stripped
5.3	Unequivocal erosion
5.4	Ulcer or granulation tissue

Follow-up

The medical records were reviewed to evaluate the clinical disease course after the baseline surveillance colonoscopy. Since a standardized follow-up using clinical disease activity scores such as the Crohn's Disease Activity Index or the Simple Clinical Colitis Activity Index was not part of the study protocol, the primary outcome of a relapse was defined as 1. step-up in IBD medication 2. hospitalization for an IBD relapse or IBD-related surgery 3. colonoscopy showing disease activity. Patients were followed from the date of the baseline surveillance colonoscopy until the development of a relapse or the last visit to the outpatient clinic.

Statistical analysis

Baseline characteristics were analysed using standard descriptive statistics. The non-parametric Mann-Whitney test was used to compare fecal calprotectin levels between patients with and without a relapse during follow-up and between patients with and without histological inflammation. The ANOVA test was used to compare fecal calprotectin levels stratified according to the maximal grade of histological inflammation according to the Geboes score and the Spearman rank correlation test was used to assess the correlation between levels of fecal calprotectin and the total score of the different Geboes parameters.

To calculate the accuracy of fecal calprotectin and the maximal Geboes score in discriminating between patients developing a relapse and those that remained in clinical remission a receiver operating characteristic (ROC) curve was constructed. The strength of the association between several clinical parameters (i.e. age, type of IBD, disease duration) and calprotectin was assessed by linear regression. Since calprotectin levels were skewed, calprotectin levels were transformed to their natural logarithm (ln calprotectin) for this analysis. A two-sided p-value <0.05 was considered statistically significant. All analyses were performed using SPSS version 20 (Chicago, IL, USA) for Windows.

Ethical considerations

This study was approved by the medical ethics committee in our center, protocol number 11-050. All participants gave written informed consent before participating in the study.

RESULTS

Patients

A total of 164 IBD patients underwent surveillance colonoscopy and provided a stool sample. Sixty-two patients were excluded because of endoscopic colonic inflammation (figure 1). Of the remaining 102 IBD patients, 11 CD patients were subsequently excluded either due to inflammation of the terminal ileum (n=5) or because intubation of the terminal ileum was not performed (n=6). A further 19 patients were excluded because no biopsies were taken from the terminal ileum in case of CD patients (n=17) or from one colonic segment (n=2). The final study population consisted of 72 patients of whom 20 patients had CD, 47 UC and five IBD-unclassified (IBD-U). Baseline characteristics of the study population are given in table 1.

Figure 1: Flow chart showing the patient selection.

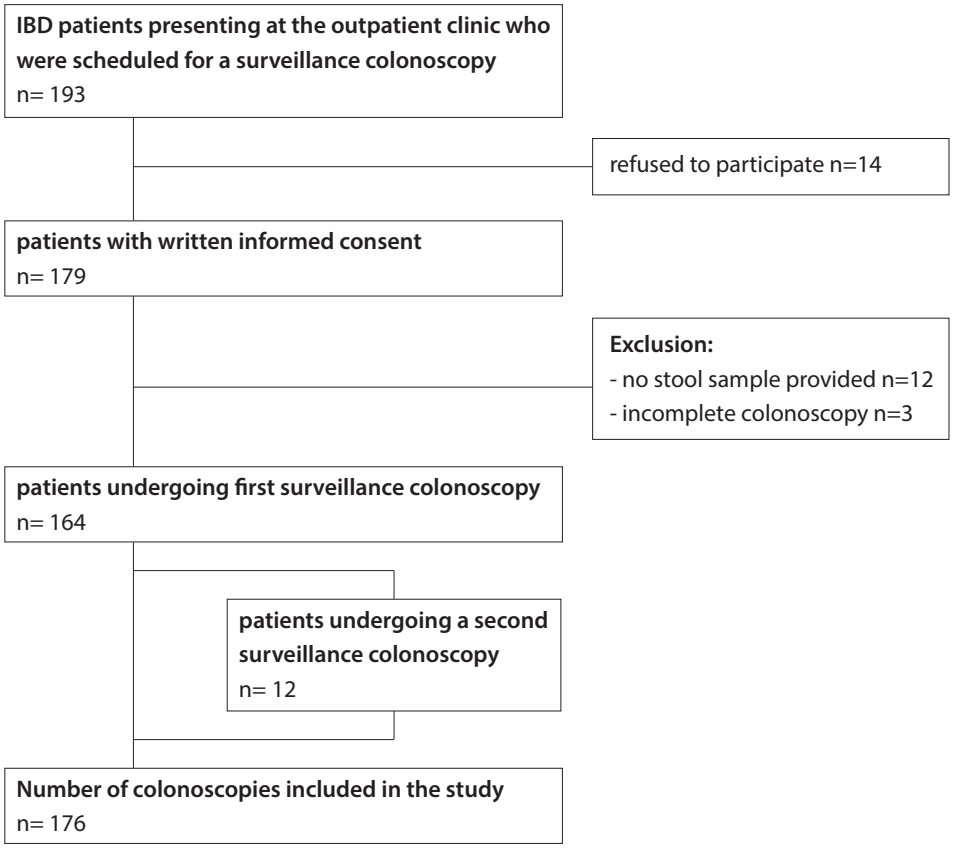


Table 1: Baseline characteristics of IBD patients undergoing surveillance colonoscopy

	N (%)
Number of patients	72 (100)
Male gender	33 (46)
Age, years (median, range)	50 (19 – 71)
Duration of IBD, years (median, range)	19 (4 – 51)
IBD diagnosis	
Ulcerative colitis	46 (64)
Left-sided	18 (39)
Extensive	25 (54)
Unknown	3 (6)
Crohn's colitis	21 (29)
Segmental colitis <50%	7 (33)
Segmental colitis >50%	14 (67)
Unknown	0 (0)
IBD-unclassified	5 (7)
Left Sided	1 (20)
Extensive	4 (80)
Unknown	0 (0)
Age at diagnosis (CD patients)	
<16 years	3 (14)
Between 17 and 40 years	15 (72)
>40 years	3 (14)
Disease phenotype (CD patients)	
Non-stricturing, non-penetrating	14 (67)
Structuring	6 (29)
Penetrating	1 (4)
Perianal disease	6 (29)
Disease location (CD patients)	
Ileal	0 (0)
Colonic	11 (52)
Ileocolonic	9 (43)
Upper GI tract	1 (5)
Medication use	
5-ASA	48 (67)
Thiopurines	32 (44)
Methotrexate	2 (3)
Anti-TNFα	5 (7)

Fecal calprotectin results

Median fecal calprotectin was 49 mg/kg (range 20 – 4609). No differences were found between CD patients (median value 53 mg/kg) and patients with UC or IBD-U (median value 43 mg/kg, p=0.35). A calprotectin level above 250 mg/kg, reportedly the optimal cut-off level for active disease, was observed in 12 patients (17%).[6]

Histological inflammation and calprotectin

Of the 72 patients, 23 (32%, 8 CD patients and 15 UC or IBD-U patients) had a Geboes score ≥3.1 in at least one biopsy, which is considered the cut-off for active histological disease in previous studies.[20] Median fecal calprotectin levels were similar for patients with active histological disease as compared to patients without active histological disease both for CD patients (45 mg/kg vs 54 mg/kg, p=0.46) and UC or IBD-U patients (74 mg/kg vs 28 mg/kg, p=0.09). After stratifying for the maximal grades of histological inflammation, no association between higher histological grades of inflammation and higher levels of fecal calprotectin was found either (p=0.27, figure 2). Furthermore, in both CD and UC or IBD-U patients, fecal calprotectin showed no significant correlation with the total score for each separate Geboes feature (table 2).

Figure 2: Calprotectin levels of patients stratified according to the maximal Geboes score. One patient with a calprotectin level of 4609 mg/kg was set at 1000 mg/kg in this graph.

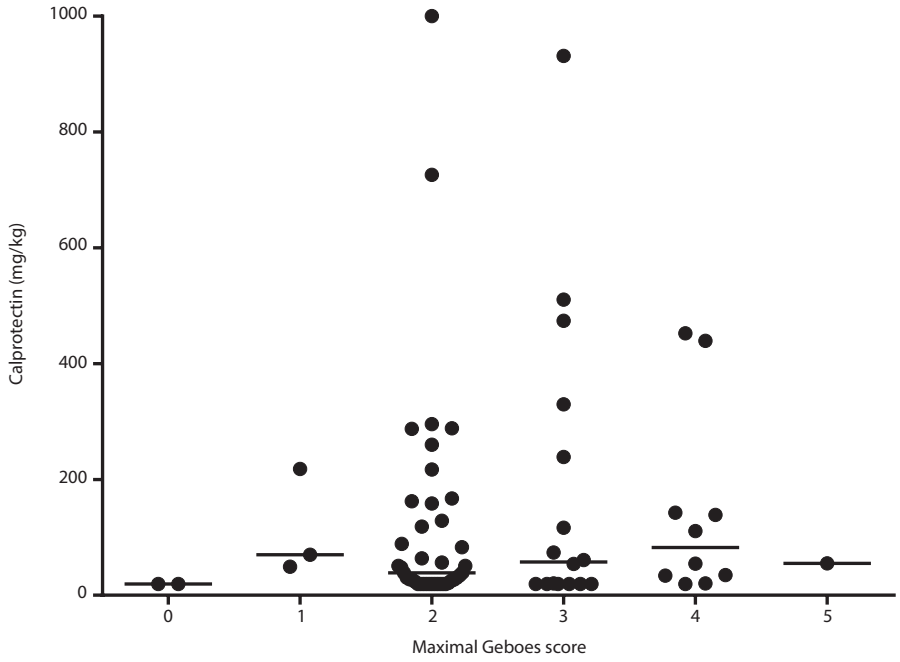


Table 2: Correlation coefficients between the histological score for each of the Geboes features and fecal calprotectin levels. All correlations have a p-value >0.05 (Spearman rank correlation test).

	Correlation coefficients	
	UC patients (53)	**CD patients (20)**
Architectural distortion	-0.11	0.21
density of inflammatory infiltrate	-0.04	0.22
density of eosinophils in the lamina propria	0.10	-0.01
density of neutrophils in the lamina propria	0.01	0.22
the presence of neutrophils in the epithelium	-0.02	0.30
crypt destruction	-0.05	0.27
mucosal damage	-0.03	NA

Clinical parameters associated with elevated calprotectin levels

Univariate analysis showed no significant association between type and duration of IBD, disease extent, age, gender, type of maintenance therapy, history of upper gastrointestinal disease, smoking, presence of post-inflammatory polyps, maximal Geboes score and the level of fecal calprotectin (table 3).

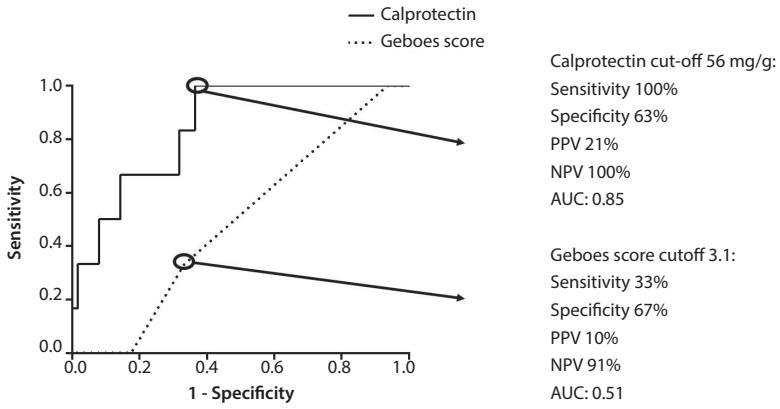
Table 3: Linear regression analysis with ln calprotectin as the dependent variable

	Regression coefficient (95% CI)	**p-value**
Male gender	-0.40 (-1.08 – 0.28)	0.44
UC and IBD-U (vs. CD)	-0.40 (-1.21 – 0.41)	0.76
Extensive colitis	0.29 (-0.37 – 0.94)	0.38
Duration of IBD, years	0.002 (-0.03 – 0.04)	0.40
Smoking	-0.41 (-1.26 – 0.45)	0.35
5-ASA use	0.50 (-0.23 – 1.23)	0.18
Thiopurine use	-0.11 (-0.73 – 0.51)	0.72
Anti-TNF use	-0.35 (-1.33 – 0.63)	0.48
History of upper gastrointestinal disease	0.48 (-0.75 – 1.72)	0.44
Maximal Geboes score	0.22 (-0.13 – 0.57)	0.21
Post-inflammatory polyps	-0.20 (-0.84 – 0.46)	0.56

Predictors of relapse during follow-up

During a median follow-up of 12 months (range 0 - 24 months), six patients (8%, 2 CD and 4 UC) relapsed. Median time between the baseline surveillance colonoscopy and relapse was 13 months (range 4 – 16 months). Three patients were started on steroids, two patients were started on 5-ASA and azathioprine dosage was optimized in one patient. Disease activity was confirmed with endoscopy in five patients showing mild or moderate colonic inflammation in all five patients. In the sixth patient, steroids were started without performing endoscopy.
Active histological inflammation at baseline, defined as a Geboes score ≥ 3.1, was observed in 2 out of 6 patients (33%) who relapsed during follow-up, and in 21 out of 66 patients (32%) who remained in clinical remission (p=0.94).
Median fecal calprotectin levels at baseline were significantly higher for patients who had a relapse during follow-up as compared to patients who remained in remission (284 mg/kg interquartile range 69 – 1697 vs. 37 mg/kg, interquartile range 20 - 131. p<0.01). Fecal calprotectin was found to be associated with an increased risk of relapse after adjusting for several potential risk factors for relapse such as maximal Geboes score, type of IBD, smoking status, gender and disease extent (adjusted hazard ratio 1,001 (95% CI 1.000 – 1.002) p=0.01).
The ROC curve showed that a baseline fecal calprotectin cut-off value of 56 mg/kg could predict a relapse during follow-up with 100% sensitivity, 63% specificity, 21% positive predictive value (PPV) 100% negative predictive value (NPV) and an area under the curve (AUC) of 0.85 (figure 3). A cut-off value of 228 mg/kg identified patients that relapsed with 67% sensitivity, 87% specificity, 31% PPV and 97% NPV. A maximal Geboes score of 3.1 or higher could predict a relapse with a 33% sensitivity, 67% specificity, 10% PPV, 91% NPV and an AUC of 0.51.

Figure 3: Receiver operator curve for fecal calprotectin and the maximal Geboes score showing the diagnostic accuracy for distinguishing between patients developing a relapse and patients remaining in clinical remission.



DISCUSSION

The therapeutic goal in IBD patients has recently shifted from symptom control to the combination with mucosal healing, because this has been found to better predict long-term remission and prevent hospitalizations and surgeries.[1-3] The present study shows that low fecal calprotectin levels in IBD patients with endoscopic mucosal healing are associated with a very low risk of relapse compared to patients with high calprotectin.

The term 'deep remission' has recently been coined to indicate a condition in which patients are in clinical, endoscopic and serological remission. For CD, this might be defined as a CDAI score of less than 150, a CRP level less than 5.0 mg/L and complete mucosal healing upon endoscopy, while for UC 'clinical remission' and 'mucosal healing' has been proposed.[16] The fact that low levels of calprotectin combined with mucosal healing further reduce the risk of relapses in the current study supports the concept of 'deep remission' and of incorporating calprotectin as a defining parameter.

We report here that calprotectin identifies patients who relapse during follow-up with 100% sensitivity and 64% specificity. This is in line with several previous studies combined in a recent meta-analysis.[13] The studies incorporated in this meta-analysis only included patients in clinical remission, however, since no attempts were made to confirm mucosal healing endoscopically. Therefore, persistent inflammation already present at baseline could explain the elevated calprotectin levels among patients that relapsed in these studies.

In the current study, a fecal calprotectin cut-off level of 56 mg/kg offered the best diagnostic accuracy for identifying patients who remained in stable remission during follow-up. This is substantially lower than the calprotectin cut-off levels reported in previous studies, ranging from 130 mg/kg to 340 mg/kg.[17-22] Since we only included patients with complete mucosal healing, this discrepancy can probably be attributed to undetected disease activity in patients in whom baseline colonoscopy was not performed. Another explanation might be that we used a different calprotectin essay, although there was an excellent correlation between this essay and the widely used Buhlmann essay.

We were unable to substantiate our hypothesis that elevated calprotectin levels in patients with endoscopic mucosal healing are caused by histological disease activity. Although 32% of patients indeed had active histological inflammation, their calprotectin levels were similar to patients without histological inflammation. This contrasts with two previous studies in which significant correlations between histological inflammation and calprotectin levels were found, although these studies both reported stronger correlations between endoscopic inflammation and calprotectin.[23, 24] These differences can be explained by exclusion of patients with any endoscopic sign of inflammation in our study, resulting in a limited range of histological inflammation.

The elevated calprotectin levels in patients in endoscopic and histological remission could also be the result of a proximal manifestation of Crohn's disease, NSAID-induced enteropathy or other inflammatory disorders of the upper gastrointestinal tract.[25, 26] Although we did not perform imaging studies to exclude upper gastrointestinal CD, we do not feel this influenced the results to a large extent since only one CD patient had a history of upper GI involvement. NSAID-use was allowed in our study, but since only three patients used NSAID's in the two weeks prior to the colonoscopy, this could only have affected our results marginally. Of note, calprotectin levels did not differ between NSAID users and non-NSAID users. Finally, sampling error might have resulted in an underestimation of the grade of histological inflammation, especially in CD patients. The same holds true for the endoscopic grading of inflammation, since the endoscopist could have missed skip lesions of inflammation potentially explaining the higher calprotectin levels. We also checked whether duration of disease, disease extent, presence of post-inflammatory polyps, medication use, smoking and history of upper gastrointestinal disease correlated with calprotectin levels in our study but this was not the case.
We could not confirm the previously reported association between histological disease activity and the risk of relapse.[27-29] This may be due to different scorings systems and the relative small number of patients developing a relapse in our study. Furthermore, only 23 patients had active histological inflammation, limiting the power of our study and therefore our claim that histological disease activity is not associated with risk of relapse. Although the Geboes scoring system is used in several previous studies, it was not previously validated as a tool to predict relapses in IBD.
In addition to the small number of patients that relapsed there are several other limitations that need to be addressed. First, although calprotectin proved to be a sensitive marker for relapses during follow-up, the low positive predictive value of 20% is a limitation and does not justify step-up therapy solely based on calprotectin levels. Second, mucosal healing was defined using the Mayo score, which is not validated for patients with CD. Third, only IBD patients undergoing surveillance colonoscopy were included in the current study and therefore these results cannot be extrapolated to the general IBD population. Fourth, we did not employ a strict follow-up regimen with clinical activity indices but instead relied on clinically evident relapses. This might have caused an underestimation of the real relapse rate since mild relapses might not have been reported by the patients.
In conclusion, this study shows that calprotectin can identify IBD patients that remain in stable remission during follow-up. Whether step-up therapy will benefit patients in remission with increased fecal calprotectin levels in the long run remains to be studied.

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Chapter 10

Fecal calprotectin testing prevents ineffective colorectal cancer surveillance procedures in patients with long-standing colitis

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Published in Inflammatory Bowel Diseases

ABSTRACT

Background

Active colitis impairs neoplasia detection during colonoscopic surveillance for colorectal cancer (CRC) in patients with inflammatory bowel disease (IBD). We investigated whether fecal calprotectin testing prior to surveillance colonoscopy might identify ineffective surveillance procedures.

Methods

All consecutive patients with Crohn's disease (CD) or ulcerative colitis (UC) scheduled for surveillance colonoscopy were asked to collect a stool sample prior to the start of bowel cleansing. Ineffective surveillance was defined as at least one colonic segment with moderate or severe inflammation. Calprotectin was analyzed using an enzyme-linked immunosorbent assay (Ridascreen®, R-Biopharm, Germany). ROC statistics were used to determine the optimal cut-off for calprotectin.

Results

A total of 176 surveillance colonoscopies were performed in 164 patients, of which 83 had CD and 81 had UC or IBD-unclassified. Complete endoscopic remission or mild inflammation categorized as effective surveillance was observed in 151 colonoscopies (86%) whereas moderate or severe inflammation categorized as ineffective surveillance was observed in 25 colonoscopies (14%). Median calprotectin levels for the effective and ineffective surveillance group were 84 mg/kg (range 20 – 4609) and 1605 mg/kg (range 66 – 26336), respectively ($p<0.01$). A cut-off of 539 mg/kg identified patients with ineffective surveillance with 84% sensitivity, 89% specificity, 55% positive predictive value, 97% negative predictive value and an area under the curve of 0.89.

Conclusion

Low fecal calprotectin accurately identifies IBD patients without colonic inflammation in whom CRC surveillance is most effective.

INTRODUCTION

Longstanding colonic inflammation in patients with Crohn's disease (CD) or ulcerative colitis (UC) increases the risk of colorectal cancer (CRC).[1, 2] For this reason, patients with a disease duration of more than eight years are advised to undergo regular endoscopic surveillance aimed at the detection of dysplasia and early stage asymptomatic CRC.[3-5] Detection of dysplasia in patients with inflammatory bowel disease (IBD) is challenging however, since dysplastic lesions are often subtle, flat or not endoscopically identifiable altogether. Chromoendoscopy has been advocated in recent guidelines, because this technique improves visualization of subtle flat lesions and thereby increases the dysplasia yield.[6-8]

If inflammation is present during surveillance colonoscopy, identification of lesions containing dysplasia is severely hampered and distinction between inflammation-associated atypia and dysplasia is challenging for pathologists.[9] This is reflected by the low interobserver agreement between pathologists for the diagnosis of indefinite and low-grade dysplasia, especially if active inflammation is present.[10, 11] Therefore, it is of paramount importance to perform CRC surveillance only in patients in complete endoscopic remission and to repeat the procedure if severe extensive inflammation is present.

Mucosal disease activity can also be present when IBD patients have no or only mild symptoms however.[12, 13] One previous study from the Netherlands showed that as much as 34% of IBD patients undergoing surveillance had endoscopic and histological signs of active inflammation despite being in clinical remission.[14] Several blood and stool-based markers have been evaluated to predict the presence of intestinal inflammation, of which fecal calprotectin seems to be the most promising. This marker was found to correlate well with endoscopic disease activity both in CD and UC patients.[15-17]

We aimed to investigate whether fecal calprotectin testing prior to surveillance colonoscopy can reliably identify patients with active inflammation, thereby preventing ineffective procedures.

METHODS

Patients

All consecutive patients with UC and CD scheduled for surveillance colonoscopy between July 2011 and August 2012 were invited to participate in the current study. Patients collected a sample of feces prior to the start of bowel cleansing and stored the sample at room temperature for a maximum of 48 hours. Patients also completed a questionnaire to collect data for the

simple clinical colitis activity index (UC patients) or the Harvey Bradshaw index without the parameter abdominal mass (CD patients) prior to the colonoscopy.
Bowel preparation was performed with 4 litres of polyethylene glycol according to the prevailing protocol in our center. Three experienced endoscopists performed the surveillance colonoscopies employing chromoendoscopy with methylene blue (0.1%). All lesions suspected of neoplasia were removed and additionally, two random biopsies were taken in four colonic segments (i.e. eight biopsies in total) to assess histological disease activity. Endoscopic disease severity and extent of inflammation were scored in all four colonic segments i.e. the ascending, transverse, descending and the rectosigmoid colon. Disease severity in each segment was scored as no inflammation, mild (erythema, decreased vascular pattern, mild friability), moderate (marked erythema, absent vascular pattern) and severe inflammation (spontaneous bleeding, ulceration) in accordance with the Mayo endoscopic score. Since CD patients could only be included if colitis was present, we decided to employ the same score for UC and CD patients in order to increase comparability between groups. Endoscopists were unaware of the calprotectin levels when performing the colonoscopies.
Patients in whom the cecum could not be reached or with insufficient bowel preparation were excluded from further analysis. If moderate or severe inflammation was present in at least one colonic segment, the procedure was categorized as ineffective surveillance. The remaining procedures in which no inflammation or mild inflammation was found were categorized as effective surveillance. The pathology reports were reviewed to assess whether any of the targeted or random biopsies contained neoplasia.

Calprotectin analysis

All stool samples were stored at -80°C directly after they were received from the patients. Calprotectin was measured with a quantitative enzyme-linked immunoassay according to the manufacturer's instructions (Ridascreen, provided by R-Biopharm, Darmstadt, Germany). Calprotectin levels were also analysed with the commonly available Buhlmann assay (Buhlmann laboratories AG, Basel Switzerland), which showed an excellent correlation with the Ridascreen results (correlation coefficient r=0.93, results not shown).

Statistical analysis

Baseline characteristics were analyzed using standard descriptive statistics. Calprotectin levels were compared between patients with effective and ineffective surveillance using the Mann-Whitney-U test. The accuracy and appropriate cut-off values of calprotectin for identifying patients with ineffective surveillance were analyzed using receiver operator characteristics (ROC) statistics. The method described by Hanley and McNeil was used to assess whether there was

a significant difference between the area under the ROC curves (AUC) of CD and UC patients.[18]
The percentage of patients diagnosed with neoplasia was compared between patients with no inflammation, mild, moderate or severe inflammation using Chi-square testing. A two-sided p-value <0.05 was considered statistically significant. Data were analysed using SPSS version 20 for windows.

Ethical considerations

This study was approved by the medical ethical committee of our institution. All patients gave written informed consent.

RESULTS

Patients

In total, 176 surveillance colonoscopies were performed in 164 patients. The recruitment of the study population is summarized in figure 1. Seventy-four patients were diagnosed with ulcerative colitis (45%), 83 had Crohn's colitis (51%) and seven had IBD-unclassified (IBD-U) (4%). Baseline characteristics of the study population are shown in table 1.
Complete endoscopic remission was observed in 111 colonoscopies (63%) while mild inflammation was present in 40 (23%), moderate inflammation in 15 (8%) and severe inflammation in 10 colonoscopies (6%)(table 2). Therefore, 151 procedures (86%) were categorized as effective surveillance and 25 procedures (14%) as ineffective surveillance.
The median SCCAI and HB score was 0 (range 0 – 15). Increased HB (>4) and SCCAI (>3) scores were observed in 12 CD patients and 12 UC or IBD-U patients. [18, 19] Of the 24 patients with increased HB or SCCAI scores, only 7 (29%) were classified as ineffective surveillance during endoscopy.

Figure 1: Flow chart summarizing the recruitment process of the study population.

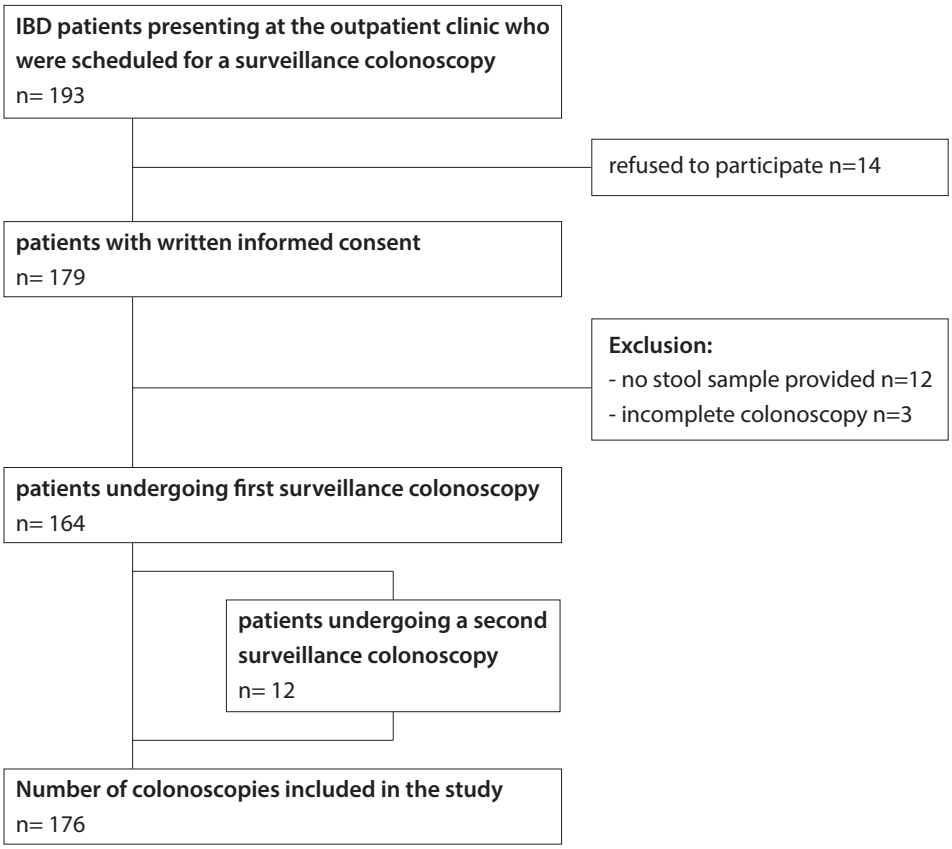


Table 1: Baseline characteristics of the patients undergoing surveillance colonoscopy

	N (%)
Number of patients	164 (100)
Male gender	81 (49)
Age, years (median, range)	49 (19 – 72)
Duration of IBD, years (median, range)	19 (1 – 51)
IBD diagnosis	
Ulcerative colitis	74 (45)
Left-sided	25 (34)
Extensive	45 (61)
Unknown	4 (5)
Crohn's colitis	83 (51)
Segmental colitis <50%	24 (29)
Segmental colitis >50%	58 (70)
Unknown	1 (1)
IBD-unclassified	7 (4)
Left Sided	3 (43)
Extensive	4 (57)
Unknown	0 (0)
Concomitant diagnosis of PSC	22 (13)
Medication use	
5-ASA	91 (55)
Thiopurines	65 (40)
Methotrexate	5 (3)
Biologicals	33 (20)
No maintenance therapy	7 (4)

Table 2: Endoscopic characteristics of the patients undergoing surveillance colonoscopy.

	N (%)
Number of surveillance colonoscopies	176 (100)
Number of biopsies (median, IQR)	
Random	9 (8 – 19)
Targeted	2 (0 – 4)
Severity of inflammation	
No inflammation	111 (63)
Mild inflammation	40 (23)
Moderate inflammation	15 (9)
Severe inflammation	10 (6)
Lesions suspected of neoplasia	206
Lesions containing neoplasia	30 (100)
Adenoma-like	24 (80)
Non-adenoma-like	6 (20)
Grade of dysplasia	
LGD	30 (100)
HGD	0 (0)
CRC	0 (0)

LGD, low-grade dysplasia; HGD, high-grade dysplasia; CRC, colorectal cancer.

Calprotectin results

Median calprotectin levels for the effective and ineffective surveillance group were 84 mg/kg (range 20 – 4609) and 1605 mg/kg (range 66 – 26336) respectively ($p<0.01$, figure 2).

ROC curve analysis showed that a cut-off level of 539 mg/kg optimally distinguished patients with effective surveillance from patients with ineffective surveillance with 84% sensitivity, 89% specificity, a positive predictive value (PPV) of 55% and a negative predictive value (NPV) of 97% (Figure 3). Overall, the diagnostic accuracy of calprotectin as reflected by the area under the ROC curve was 0.89. These results were similar for patients with UC as compared to CD (AUC 0.86 vs 0.92, respectively, $p=0.42$).

If the calprotectin cut-off level of 539 mg/kg would be applied to the study population, 38 patients (22%) had levels above the cut-off of which 21 (55%) were classified as ineffective surveillance and 17 (45%) as effective surveillance. Of the 138 patients with levels below the cut-off, only 4 patients (3%) were classified as ineffective surveillance. Therefore, calprotectin testing prior to a scheduled surveillance colonoscopy could potentially reduce the incidence of ineffective surveillance due to inflammation from 25 procedures to four procedures (14% to 3%, $p<0.01$) with a number needed to test to identify one patient with ineffective surveillance of 8.4.

Figure 2: Calprotectin levels of patients classified as effective and ineffective surveillance. The median calprotectin level was 84 mg/kg (range 20 – 4609) for the effective surveillance group and 1605 mg/kg (range 66 – 26336) for the ineffective surveillance group (p<0.01).

Four patients in the ineffective surveillance group had calprotectin levels above 5000 mg/kg and were set at a maximum of 5000 mg/kg in this graph.

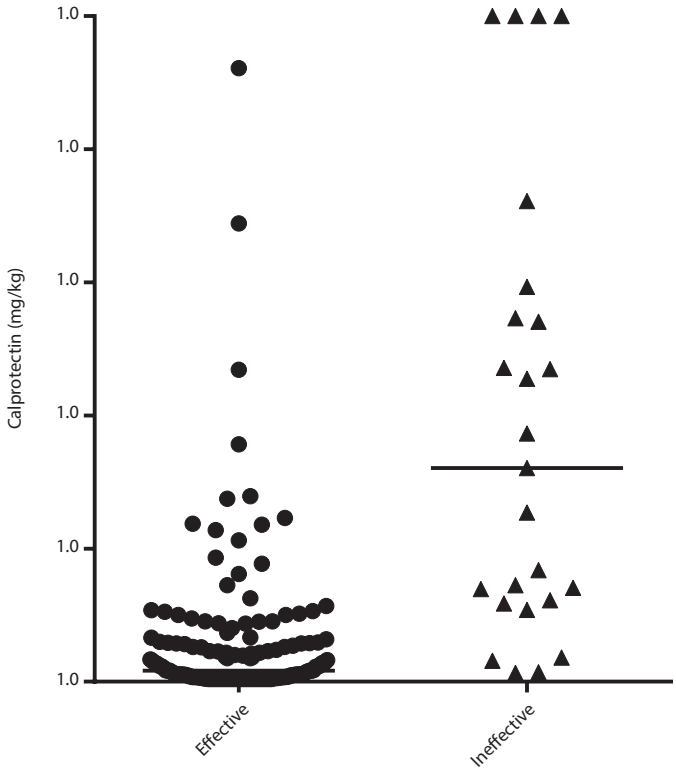
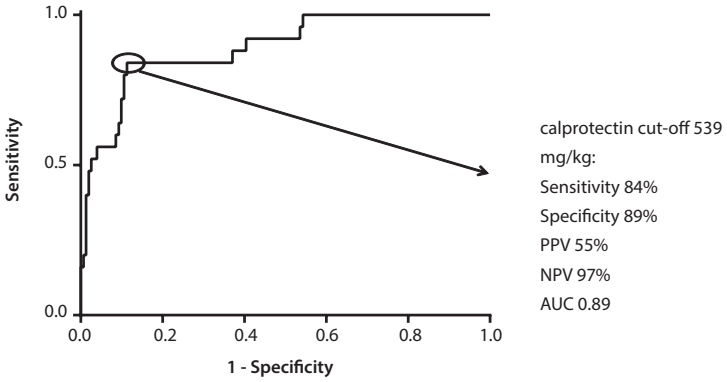


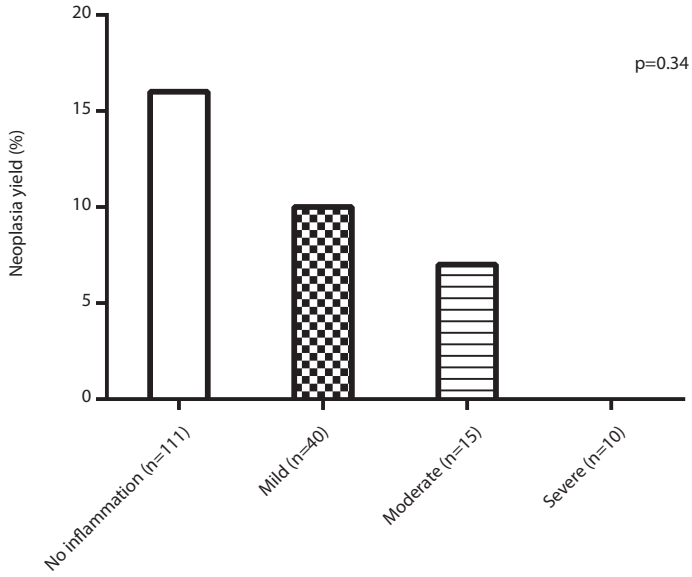
Figure 3: Receiver operator curve showing the diagnostic accuracy of fecal calprotectin in distinguishing between patients with effective and ineffective surveillance. Ineffective surveillance was defined as patients with moderate or severe inflammation.



Dysplasia detection

During a total of 176 surveillance colonoscopies, 30 dysplastic lesions were detected in 23 colonoscopies (13%). Two patients were diagnosed with low-grade dysplasia (LGD) detected in a random biopsy. The remaining 28 dysplastic lesions were diagnosed in endoscopically visible lesions which were classified by the endoscopists as adenoma-like in 24 lesions and as non-adenoma-like in the remaining four lesions (table 2). The adenoma-like lesions were histologically classified as tubular adenomas (n=21) serrated adenomas (n=2) and tubulovillous adenoma (n=1) all containing LGD. The 4 non-adenoma-like lesions contained LGD in all four cases. Among the 111 surveillance colonoscopies with no inflammation the neoplasia yield was 24 dysplastic lesions in 18 colonoscopies (16%). In 4 out of 40 colonoscopies with mild inflammation 5 dysplastic lesions were detected (10%). Only one dysplastic lesion in 15 colonoscopies with moderate inflammation (7%) and no dysplastic lesions in 10 colonoscopies with severe inflammation (0%) was found (p=0.34, figure 4). The dysplasia yield among the 151 patients with effective surveillance was 15% compared to 4% among the 25 patients with ineffective surveillance (p=0.15).

Figure 4: The percentage of patients diagnosed with neoplasia during the surveillance colonoscopies stratified according to the maximal degree of inflammation present during the procedure.



DISCUSSION

Endoscopic surveillance in patients with longstanding colonic IBD is challenging because IBD-associated dysplasia is more difficult to detect than sporadic adenomas in patients without IBD. Good bowel preparation, optimal visualization with chromoendoscopy and an absence of mucosal disease activity are therefore of utmost importance to optimize the effectiveness of this procedure and increase dysplasia detection rates. This study shows that fecal calprotectin testing prior to a scheduled surveillance colonoscopy can significantly reduce the number of ineffective procedures, defined as colonoscopies showing persistent moderate or severe inflammation from 14% to 3%.

The large proportion of active inflammation encountered among patients undergoing surveillance in our study (37% including patients with mild inflammation) is in line with previous studies, which reported endoscopic inflammation in 34% to 50% of cases, despite the fact that these patients were in clinical remission.[14, 21] This is most likely because clinical symptoms do not accurately predict mucosal inflammation in both CD and UC patients.[12, 13]

Although our results show that a cut-off of 539 mg/kg of calprotectin can predict ineffective

surveillance, we did not investigate what the best management is when calprotectin is above this threshold. Baars et al. showed that a short course of corticosteroids prior to a surveillance colonoscopy might decrease histological disease severity, although the decrease in endoscopic disease severity was not statistically significant.[22] Postponing the procedure and initiating a step-up in maintenance therapy to achieve mucosal healing seems a logical approach, although this was not the scope of the present study.

We also reported a lower dysplasia yield as the severity of inflammation found during surveillance increased. Although not statistically significant, the dysplasia yield decreased from 16% in patients with complete mucosal healing to 0% in patients with severe inflammation. This seems in contrast to the commonly accepted concept that persistent inflammation in IBD is a risk factor for the development of colitis-associated carcinoma.[23-25] We believe this can be attributed to the difficulty of detecting dysplasia in inflamed mucosa. Missed lesions and the carcinogenic effect of ongoing inflammation most likely explain the increased risk of neoplasia in these cases over time as reported by previous studies.

Analysis of one calprotectin sample costs around 30 euros (40 dollars) as compared to 459 euros (623 dollars) for colonoscopy. Based on our study cohort, calprotectin testing would have cost 5,280 euros (7,161 dollars) and saved 9,639 euros (13,074 dollars) in colonoscopy costs. Therefore, the costs of calprotectin testing prior to a surveillance colonoscopy seem to be balanced out by the cost savings of reducing the number of ineffective surveillance procedures.

Our study has several limitations that need to be addressed. The low PPV of 55% implies that the surveillance procedure would be incorrectly postponed in 45% of cases with calprotectin levels above the cut-off of 539 mg/kg. Therefore, postponing the procedure and initiating a step-up in therapy cannot be based solely on an elevated calprotectin level, which limits its usefulness in clinical practise.

Due to the low incidence of dysplasia we were only able to identify trends regarding differences in dysplasia yield between patients with effective and ineffective surveillance. Although the strategy of fecal calprotectin testing was able to significantly reduce the number of ineffective surveillance procedures, the strategy of postponing all surveillance colonoscopies when calprotectin is above the cut-off would have resulted in a nonsignificant increase in dysplasia yield from 13% to 15% in our study population (p=0.64).

Therefore, we cannot definitively conclude that calprotectin testing prior to a surveillance colonoscopy increases the dysplasia yield and therefore the effectiveness of the procedure.

There is no well-defined cut-off to determine when a surveillance procedure is ineffective due to inflammation. Current surveillance guidelines simply state that surveillance colonoscopies, whenever possible, should be performed when the disease is in remission.[4] We chose to

classify patients with moderate and severe inflammation as ineffective procedures and mild or no inflammation as effective. The gradually decreasing neoplasia yield as the severity of inflammation increases would suggest that the neoplasia yield might be even better when patients with mild inflammation were also classified as ineffective surveillance. If patients with mild inflammation would have been classified as ineffective surveillance as well, 65 procedures (37%) would have been ineffective. Using a lower cut-off of 140 mg/kg calprotectin would have identified 56 of these patients (86%). Regarding the dysplasia yield, postponing all these procedures would increase the dysplasia yield only marginally to 16% as compared to 15% when only patients with moderate or severe inflammation were classified as ineffective. Since 40 patients were diagnosed with mild inflammation leading to a considerable rise in the number of procedures that need to be postponed, we feel that a cut-off at moderate or severe inflammation offers the best compromise between the number of procedures that need to be postponed and the increase in neoplasia yield.

The decision to abort the surveillance colonoscopy and repeat the procedure after induction therapy was left at the discretion of the endoscopists in the current study population, rather than the aforementioned cut-off of patients with moderate or severe inflammation. Therefore, the procedure was repeated in only seven patients of the 25 classified as ineffective surveillance. Because the endoscopists knew that the procedure would be repeated their effort to detect neoplasia was probably lower which could explain the lower yield in neoplasia in patients with inflammation. If these seven patients were excluded, neoplasia yield changed from 8% to 7% for the patients with moderate inflammation and remained 0% for the patients with severe inflammation.

In conclusion, low fecal calprotectin can accurately identify IBD patients without active inflammation in which CRC surveillance is most effective. Future studies should focus on whether the routine use of this tool and postponing surveillance procedures based on the calprotectin level is cost effective.

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Chapter 11

Summary and discussion

Summary and discussion

The two main types of inflammatory bowel disease, ulcerative colitis (UC) and Crohn's disease (CD), are characterized by relapsing episodes of inflammation throughout the gastrointestinal tract. Chronic inflammation of the colonic mucosa is associated with an increased risk of colorectal cancer (CRC) in patients with IBD.[1,2] Endoscopic surveillance aimed at the detection of dysplasia and asymptomatic CRC in a curable stage is therefore recommended in order to mitigate this risk.[3,4] Mainly due to the fact that surveillance was implemented before randomized trials demonstrated its benefits, solid evidence that surveillance is effective is not available.[5] In part 1 of this thesis we critically explored various aspects of endoscopic surveillance in patients with IBD.

As IBD is characterized by relapsing episodes of inflammation, constant monitoring for the presence of inflammation is vital in evaluating response to treatment. This stimulated the search for novel non-invasive markers reliably reflecting outcomes of ileocolonoscopy, which is currently considered the gold standard test to evaluate inflammatory activity. In part 2 of this thesis we aimed to study the value of fecal calprotectin, a promising and relatively new marker for inflammation, in the setting of IBD.

Part 1: Surveillance strategies for colorectal cancer in inflammatory bowel disease

Although it is widely acknowledged that patients with longstanding colitis have an increased risk of developing CRC, the magnitude of this risk is still a matter of debate.[6] A frequently cited meta-analysis including 116 studies and close to 55.000 IBD patients concluded that the cumulative risk of developing CRC is as high as 18% after 30 years of disease duration.[7] Our group published a recent update of this meta-analysis showing lower incidence rates and highlighting that the higher incidence of CRC mainly originated from referral center cohorts.[8] Using a nationwide cohort of patients with IBD-associated CRC, we were able to demonstrate in **chapter 2** that CRC patients treated in referral centers had a more severe course of IBD prior to the CRC diagnosis. Since extent and severity of inflammation are risk factors for IBD-associated CRC, the more severe IBD phenotype among referral center patients might well explain the higher incidence of CRC in these centers. Interestingly, the more severe course of IBD did not result in a shorter interval between the IBD and CRC diagnosis. This suggests that although the risk of CRC increases with a greater extent and severity of inflammation, this does not seem to accelerate the inflammation-dysplasia-carcinoma sequence in these patients.

Endoscopic surveillance is aimed at detecting dysplasia or early stage asymptomatic CRC and thereby ultimately reducing CRC-related mortality. The effectiveness of this strategy critically depends on the sensitivity of the screening tool (i.e. the ability to detect dysplasia with colonoscopy) and the surveillance interval. The first surveillance guidelines were published in

2002 by the British Society of Gastroenterology (BSG) and the American Gastroenterological Association (AGA).[9, 10] In short, both guidelines recommended regular surveillance for all patients with extensive colitis after 8 years of disease duration at an interval of 1-2 years. A potential drawback of a longer interval between two surveillance procedures is the increased risk of CRC either developing from dysplasia missed during the previous surveillance procedure or the rapid development of a new CRC, also known as interval CRC. In **chapter 3** we showed that the overall incidence of these interval CRCs was low among IBD patients undergoing regular endoscopic surveillance at the appropriate interval of 1-2 years. Although this low incidence might justify longer surveillance intervals in order to decrease costs and burden for the patient, the fact that interval CRCs still comprised one third of the total number of CRCs do not support this claim. Unfortunately, we could not identify risk factors for interval CRC due to the low number of interval CRCs in our cohort.

Both surveillance guidelines were updated in 2010, but only the BSG guidelines changed the recommended surveillance intervals to 1, 3 or even 5 years depending on the presence of several well-known risk factors for CRC.[3, 4] The updated AGA guideline mentioned the same risk factors, but still maintains the recommendation to perform surveillance every 1-3 years. In **chapter 4** we reported that the longer surveillance intervals of the new BSG guidelines resulted in a reduction of the colonoscopic workload by 22% compared to the AGA guidelines when applied to a large cohort of IBD patients undergoing surveillance. The estimated number of patients developing dysplasia or CRC was similar between the three risk groups of the BSG guidelines, whereas the high-risk group of the AGA guideline including patients with prior dysplasia or PSC may be at higher risk of dysplasia or CRC. These results highlight the need for future studies aimed at improving the identification of patients at highest risk of developing CRC. Our group previously reported that a clinical prediction rule combining four clinical risk factors and biomarkers in colonic biopsies of patients with low-grade dysplasia such as p53 and AMACR has a reasonably good predictive power for CRC during follow-up.[11, 12] The findings of these retrospective case-control studies need to be validated in prospective studies, including both the confirmation of known risk factors and the identification of new factors to provide better risk stratification and thereby targeting surveillance to those at highest risk and most likely to benefit.

In contrast to the adenoma-carcinoma sequence observed in the development of sporadic CRC, IBD-associated CRC develops along an inflammation-dysplasia-carcinoma sequence. In addition to the different carcinogenesis, a wide variety of endoscopic appearances of dysplastic lesions can be observed in IBD patients. Traditionally, it was believed that any visible lesion containing histologically confirmed dysplasia, also known as a "dysplasia associated lesion or mass" (DALM), was associated with a high risk of synchronous of metachronous cancer and

should therefore be treated with colectomy.[13] Recent studies have shown however that the sub-group of lesions with an endoscopic appearance resembling that of sporadic adenomas (subcategorized as adenoma-like DALM) also found in patients without IBD have a lower risk of CRC than previously perceived and can therefore be managed endoscopically.[14, 15] In **chapter 5**, we reported that for IBD patients with an adenoma-like DALM at baseline, the incidence of HGD or CRC during follow-up was higher compared to IBD patients without adenoma and non-IBD patients with an adenoma. Although the overall risk of CRC was still quite low at 5%, these results suggest that intensified surveillance is indicated in patients following resection of an adenoma.

The various categories of dysplastic lesions encountered in IBD patients combined with a variety of non-dysplastic lesions can be quite confusing for the endoscopist and make IBD surveillance colonoscopies a challenging procedure. In addition, there are no distinct histological features that can aid in the differentiating between adenoma-like and non-adenoma-like DALM's. Therefore, we investigated the ability of endoscopists to differentiate dysplastic from non-dysplastic lesions as well as the inter-observer variability for categorizing lesions as adenoma-like and non-adenoma-like DALM's in **chapter 6**. The results were rather disappointing with a sensitivity and specificity for differentiating dysplastic from non-dysplastic lesions of 74% and 54%. Combined with a low inter-observer agreement (kappa value of 0.21) on which lesions should be classified as non-adenoma-like DALM and adenoma-like DALM, these results underline the limitations of classifying lesions based on endoscopic characteristics. Since there are no clear histological features which can aid in this differentiation either, we propose to abandon the classification of lesions as adenoma or non-adenoma like.[16] We support the new classification proposed in the recent ECCO guideline that states that lesions appearing to be endoscopically resectable should be treated as such by an expert endoscopist, irrespective of whether the lesion is deemed adenoma-like or non-adenoma-like during endoscopy. If complete resection is achieved and the biopsies of the surrounding mucosa are negative for dysplasia, annual surveillance is recommended thereafter although the optimal surveillance interval after removal of a dysplastic lesion remains a matter of debate.

Several studies have demonstrated that dye-based chromoendoscopy using methylene blue or indigo carmine aimed at highlighting subtle changes in the pit pattern can increase the dysplasia detection rates compared to white-light endoscopy with random biopsies.[17-20] In light of this evidence, both the BSG and ECCO guidelines recommend chromoendoscopy as the preferred surveillance method, although the AGA guidelines only recommend the use of chromoendoscopy for physicians with sufficient experience with the technique. In **chapter 7** we presented data of our experience with chromoendoscopy, when used as the standard surveillance method. In contrast to the randomized trials, we did not find an increase in

neoplasia detection after the implementation of chromoendoscopy as compared to the conventional white light endoscopy plus random biopsies protocol. Although more studies are needed to confirm this, these results cast doubt on the standard use of chromoendoscopy as the preferred surveillance tool in IBD. Since the dysplasia yield remained comparable even when the dysplasia detected using random biopsies was excluded, our results suggest that the future of surveillance could potentially be white light endoscopy without random biopsy sampling or the use of chromoendoscopy.

Part 2: Biomarkers for inflammation in inflammatory bowel disease

In part 2 of this thesis we focused on the role of calprotectin, a fecal biomarker for inflammation in IBD. In **chapter 8** we reported that fecal hemoglobin had a similar diagnostic accuracy for the detection of inflammation as calprotectin in patients with CD and UC. Although the combination of both tests did not show a significant improvement in diagnostic accuracy compared to each marker alone, sequential testing increased the probability of detecting inflammation from 64% to 78% when both markers were elevated compared to calprotectin testing alone.

In **chapter 9** we reported that low calprotectin levels are associated with sustained clinical remission during follow-up in IBD patients with complete mucosal healing. These results support the concept of trying to achieve "extreme deep remission" defined as clinical and endoscopic remission in combination with a low calprotectin level as the risk of relapse was 0% in patients in whom all these characteristics were present. However, the low positive predictive value means that elevated calprotectin in patients with mucosal healing is probably not enough to justify a step up in medication. We also concluded that low-grade inflammation that is only detectable histologically did not explain the higher calprotectin levels and the subsequent increased risk of relapse in patients with mucosal healing. This finding is not compatible with the fact that the amount of neutrophils present in the biopsies, which are known to contain calprotectin, form an integral part of the Geboes histological scoring system for inflammation. Although the discontinuity of inflammation ('skip lesions') missed by the biopsies could explain this discrepancy, the question as to where the high levels of calprotectin originate from in these specific cases remains to be answered.

Surveillance for CRC in patients with IBD is a challenging procedure requiring optimal bowel preparation and absence of inflamed mucosa in order to detect subtle dysplastic lesions, especially when using chromoendoscopy. In **chapter 10** we reported that low fecal calprotectin can accurately identify IBD patients without active inflammation in which CRC surveillance is most effective. Using a high cut-off level for calprotectin of 539 mg/kg, the number of ineffective procedures could be reduced from 14% to 3%. Since the ineffective surveillance procedure needs to be repeated, calprotectin testing prior to a surveillance colonoscopy has the potential to both decrease the costs as well as burden for the patient.

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Chapter 12

Summary in Dutch / Nederlandse samenvatting

SAMENVATTING

De twee belangrijkste vormen van inflammatoire darmziekten ('inflammatory bowel disease' of IBD) zijn colitis ulcerosa (CU) en de ziekte van Crohn (ZvC) die zich kenmerken door terugkerende episodes van ontsteking van het spijsverteringskanaal. De chronische ontsteking van de dikke darm is geassocieerd met een verhoogd risico op het ontstaan van colorectaal carcinoom (CRC) bij patiënten met IBD. Endoscopische surveillance middels een coloscopie op vaste intervallen wordt aanbevolen om dit risico te verminderen. In deel 1 van dit proefschrift kijken we kritisch naar de huidige manier waarop surveillance wordt uitgevoerd.
IBD kenmerkt zich door periodes waarin de ontsteking opvlamt. Daarom is het belangrijk om de ontstekingsactiviteit van de darm te kunnen meten als een patiënt meer klachten heeft. Dit kan alleen middels een coloscopie, wat een invasief en belastend onderzoek is. Deel 2 van dit proefschrift beschrijft enkele studies naar het gebruik van calprotectine, een stof die gemeten kan worden in ontlasting en een goede overeenstemming vertoont met de ontstekingsactiviteit bij coloscopie.

Deel 1: surveillance strategieën voor colorectaal carcinoom bij patiënten met inflammatoire darmziekten

Hoewel er consensus bestaat dat een langdurige ontsteking van de dikke darm het risico op CRC verhoogd bij patiënten met IBD, is er nog wel discussie in welke mate dit risico verhoogd is. Een vaak geciteerde meta-analyse die de resultaten van 116 studies en 55.000 IBD patiënten combineerde, rapporteerde een cumulatief risico van 18% op CRC bij een ziekteduur van 30 jaar. Onze onderzoeksgroep publiceerde een vervolg op deze meta-analyse waaruit bleek dat de kans op CRC lager was en dat de hogere CRC incidentie met name afkomstig was uit studies van academische patiënten. Met een database van IBD patiënten met CRC uit heel Nederland konden we in **hoofdstuk 2** laten zien dat de CRC patiënten die in een academisch centrum behandeld waren inderdaad een ernstiger ziektebeloop hadden van de IBD voorafgaand aan de diagnose CRC. Omdat de uitbreiding en ernst van de ontsteking ook belangrijke risicofactoren zijn voor het ontwikkelen van CRC, is het goed mogelijk dat dit ernstiger ziektebeloop een verklaring is voor het hogere risico op CRC van patiënten in academische centra.
Endoscopische surveillance is gericht op het ontdekken van dysplasie (een voorloper van CRC) en CRC in een vroeg stadium waarop het nog geen klachten geeft en daarmee het verlagen van de mortaliteit gerelateerd aan CRC. De effectiviteit van deze strategie is afhankelijk van de sensitiviteit van het screeningsonderzoek (het vermogen om dysplasie en CRC op te sporen middels een coloscopie) en met welk interval het screeningsonderzoek wordt toegepast. Een potentieel nadeel van een langer surveillance interval is immers een hoger risico op het ont-

staan van CRC tussen 2 surveillance scopieën in, wat ook wel een interval CRC wordt genoemd. In **hoofdstuk 3** laten we zien dat de incidentie van deze interval CRC's laag is binnen een grote groep IBD patiënten die surveillance ondergingen met een interval van 1-2 jaar. Deze lage incidentie van interval CRC's kan gezien kan worden als een argument voor langere surveillance intervallen, hoewel het feit dat een derde van de gevonden CRC's geclassificeerd konden worden als interval CRC wel de noodzaak tot het vaststellen van een surveillance interval dat afhankelijk is van het risico op CRC onderstreept.

De meest gevolgde richtlijnen voor surveillance zijn afkomstig van de Britse vereniging voor gastro-enterologie (BSG) en de Amerikaanse gastro-enterologie vereniging (AGA) en zijn in 2010 aangepast. De BSG richtlijn heeft hierbij de surveillance intervallen aanpast naar 1,3, of 5 jaar, afhankelijk van de aanwezigheid van bekende risicofactoren voor CRC. De nieuwe AGA richtlijn noemt dezelfde risicofactoren, maar houdt vast aan een surveillance interval van 1-3 jaar. In **hoofdstuk 4** rapporteren we dat het aantal uit te voeren surveillance coloscopieën met 22% daalt als de risicostratificatie van de BSG richtlijn wordt toegepast op een cohort IBD patiënten die surveillance dienen te ondergaan in vergelijking met de AGA richtlijn. Het aantal patiënten dat dysplasie of CRC ontwikkelde was echter gelijk tussen de 3 groepen van de BSG richtlijn, terwijl er wel een hoger risico op dysplasie of CRC was in de hoog risico groep van de AGA richtlijn.

Bij patiënten zonder IBD ontstaat CRC uit een adenoom. Bij IBD patiënten treden onder invloed van ontsteking allerlei genetische veranderingen op die aanleiding geven tot het ontstaan van dysplasie, van waaruit uiteindelijk CRC ontstaat. Dysplasie kent vele verschijningsvormen bij patiënten met IBD, waarbij onderscheid gemaakt wordt tussen een laesie die lijkt op het adenoom wat ook bij patiënten zonder IBD voorkomt en een zogenoemde niet-adenomateuze laesie. Eerdere studies hebben laten zien dat een laesie die lijkt op een adenoom bij IBD patiënten geen extra verhoogd risico op het ontwikkelen van CRC geeft. In **hoofdstuk 5** laten we echter zien dat IBD patiënten met een adenoom wel degelijk een verder toegenomen risico hebben op het ontstaan van hooggradige dysplasie CRC in vergelijking met IBD patiënten zonder adenoom en niet-IBD patiënten met een adenoom. Hieruit concludeerden we dat een adenoom bij patiënten met IBD aanleiding moet zijn tot het intensiveren van de surveillance.

Het endoscopisch onderscheiden van verschillende types dysplastische laesies alsmede een verscheidenheid aan niet dysplastische laesies maakt een surveillance coloscopie een lastige procedure. In **hoofdstuk 6** onderzochten we middels een digitale vragenlijst met endoscopische plaatjes hoe goed endoscopisten deze verschillende soorten laesies konden onderscheiden. Hieruit bleek dat endoscopisten met een redelijke nauwkeurigheid dysplastische van niet dysplastische laesies kunnen onderscheiden, maar dat er een lage overeenstemming is

wat betreft de classificatie van verschillende subtypes van dysplastische laesies. Dit pleit voor een nieuwe en simpelere manier van indelen van dysplastische laesies waarbij alleen gekeken wordt of de laesie endoscopisch te verwijderen is of niet, ongeacht hoe deze laesie eruit ziet. Recent hebben een aantal gerandomiseerde studies laten zien dat het toepassen van chromoendoscopie, waarbij het slijmvlies van het colon blauw gekleurd wordt met een kleurstof om subtiele dysplastische laesies beter zichtbaar te maken, ervoor zorgt dat er meer dysplasie gevonden wordt. In tegenstelling tot deze gerandomiseerde studies, concluderen wij in **hoofdstuk 7** dat chromoendoscopie in de klinische praktijk niet heeft geleid tot een toename in de detectie van dysplasie nadat dit als de standaard surveillance methode is ingevoerd in 3 academische ziekenhuizen. Hoewel er meer studies nodig zijn om deze bevinding te bevestigen, suggereert dit dat chromoendoscopie geen voordeel geeft ten opzichte van het huidige protocol en daarom niet als standaard methode aanbevolen moet worden.

Deel 2: biomarkers voor ontsteking bij inflammatoire darmziekten

Deel 2 van dit proefschrift bevat de resultaten van enkele studies naar calprotectine, een stof die gemeten kan worden in de ontlasting en een goede correlatie toont met de ernst en de uitbreiding van de ontsteking in het colon. In **hoofdstuk 8** rapporteren we dat hemoglobine gemeten in ontlasting bij patiënten met IBD een even goede voorspeller is voor ontsteking als calprotectine. Hieruit concludeerden we dat het meten van hemoglobine in de ontlasting een goed alternatief is voor het meten van calprotectine, waarbij het testen van zowel calprotectine als hemoglobine de sensitiviteit verhoogde van 64% tot 78% in vergelijking met alleen calprotectine.

In **hoofdstuk 9** lieten we zien dat een laag calprotectine bij IBD patiënten die endoscopisch in complete remissie zijn geassocieerd is met een langdurig gunstig klinisch beloop zonder opvlammingen van ontsteking. We concludeerden hieruit dat het wellicht noodzakelijk is om met behandeling niet alleen naar endoscopische remissie te streven, maar de behandeling te intensiveren totdat ook het calprotectine laag is.

Een surveillance coloscopie gericht op het opsporen van afwijkingen met dysplasie in het colon is een lastige procedure. Het is essentieel dat de darmvoorbereiding goed gedaan wordt maar ook dat er geen ontstoken slijmvlies is omdat dit de detectie van afwijkingen lastiger maakt. In **hoofdstuk 10** rapporteerden we dat een bepaling van calprotectine voorafgaand aan een geplande surveillance scopie gebruikt kan worden om te voorspellen of de procedure nog nuttig is of afgezegd dient te worden vanwege teveel ontstekingsactiviteit.

Conclusie en vooruitblik

In deel 1 van dit proefschrift concludeerden we dat langere surveillance intervallen zoals beschreven in de nieuwe BSG richtlijn de voorkeur verdienen gezien de afname in het aantal coloscopieën en de lage incidentie van CRC. Het hoge percentage interval CRC's benadrukt echter wel de noodzaak tot een goede risicostratificatie, waarbij wij lieten zien dat de stratificatie zoals voorgesteld door de nieuwe BSG richtlijn onvoldoende effectief is. Er zijn dus meer prospectieve studies nodig om bekende risicofactoren te kunnen valideren en nieuwe factoren te kunnen identificeren.

We concludeerden ook dat de invoering van chromoendoscopie niet resulteerde in een toegenomen dysplasie detectie. Er zijn dus nieuwe vergelijkende studies nodig naar de optimale surveillance methode. Wellicht dat met de huidige kwaliteit coloscopen en met meer aandacht voor een goed voorbereid colon zonder ontstekingsactiviteit aanvullende technieken om dysplasie te detecteren zoals chromoendoscopie overbodig maakt. We toonden daarnaast aan dat het bepalen van calprotectine voorafgaand aan een surveillance coloscopie kan voorkomen dat de procedure vanwege te veel ontstekingsactiviteit herhaald moet worden.

Daarnaast rapporteerden we dat de huidige classificatie van dysplastische laesies bij IBD surveillance verwarrend is voor endoscopisten. Dit pleit voor een andere indeling, waarbij alleen gedifferentieerd wordt tussen laesies die wel of niet endoscopisch resectabel zijn.

In deel twee van dit proefschrift bevestigden wij eerdere studies die tonen dat calprotectine gemeten in de ontlasting een goede voorspeller is voor ontsteking in het colon en tonen we dat hemoglobine een goede alternatieve voorspeller is. Daarnaast rapporteerden we dat een laag calprotectine bij IBD patiënten een langdurige klinische remissie voorspelt. Dit ondersteunt het routinematig bepalen van calprotectine om het ziektebeloop te monitoren. Grotere prospectieve studies zullen echter moeten uitwijzen of calprotectine gebruikt kan worden als een parameter om te besluiten tot een intensivering van de behandeling.

Chapter 13

Dankwoord

DANKWOORD

Onderzoek doen is teamwork. Hierbij wil ik iedereen die mij heeft geholpen of ondersteund tijdens mijn promotietraject persoonlijk bedanken.

Allereerst alle patiënten die bereid waren deel te nemen aan de 'surveillance studie'. Zonder jullie bijdrage aan de wetenschap was een belangrijk deel van dit werk nooit tot stand gekomen.

Beste professor Siersema. Hoewel de dagelijkse begeleiding bij Bas lag, heb ik uw scherpe vragen tijdens ons maandelijks overleg altijd weten te waarderen. U zorgde voor overzicht tijdens mijn promotietraject en aansporing op het juiste moment. Veel dank voor uw begeleiding de afgelopen 4 jaar.

Dr. B. Oldenburg, beste Bas. Wat begon met een mail voor een onderzoeksstage in 2009 is geëindigd in dit mooie boekwerk. Gedurende de afgelopen jaren heb ik geen moment spijt gehad van mijn keuze voor promotieonderzoek, mede door jouw uiterst plezierige begeleiding. Tussen de poli's en scopieën door was er altijd tijd om te brainstormen. Je wist steeds een duidelijke lijn te ontdekken in de stapel tabellen en figuren die ik meebracht en je enthousiasme over de uitkomsten is aanstekelijk. Ik kijk uit naar de nieuwe samenwerking in de kliniek tijdens mijn opleiding tot MDL arts.

Alle leden van de beoordelingscommissie, prof. dr. G.J.A. Offerhaus, prof. dr. E.E.S. Nieuwenhuis, prof. dr. G. Dijkstra, Prof. dr. I.M. Hoepelman, prof. dr. C.E. Hack, veel dank voor het beoordelen van dit proefschrift.

Dr. M.E.I.S. Schipper, beste Marguerite. Heel veel dank voor het bekijken van alle coupes voor hoofdstuk 9. Onze gesprekken over de tour de france en je enthousiaste uitleg over wat er allemaal te zien was in de biopten maakte het soms ietwat saaie werk tot een wekelijks uitje.

Dr. M.G.H. van Oijen, beste Martijn. Met name aan het begin van mijn onderzoekstraject heb jij mij geholpen met de statistiek en de opzet van de eerste studies. Ik heb veel van je geleerd, ondanks dat je helaas al snel richting LA vertrok.

En dan alle collega onderzoekers, wat zou mijn promotietraject saai zijn geweest zonder jullie! Allereerst **Jan-Steven**, de pater familias van de onderzoekers. Altijd in voor een gesprek over de problemen van de dikke medemens, schrijver van bijzondere sinterklaas gedichten.

Wat goed dat je via een omweg toch een mooie plek in opleiding tot internist hebt weten te bemachtigen. **Mirthe,** wat zouden al die borrels en feestjes geweest zijn zonder jou? Ook al beïnvloedde dit de wetenschappelijke output soms, ik ben ervan overtuigd dat je promotie uiteindelijk goed gaat komen. Jammer dat je naar het Nijmeegse vertrokken bent. **Fiona,** lang geleden is het allemaal begonnen met mijn wetenschappelijke stage onder jouw begeleiding. Ik vond de samenwerking erg plezierig en heb veel van je geleerd tijdens onze besprekingen over het poliepenstuk. Je snelheid met lopen, promoveren en publiceren heb ik nooit kunnen evenaren, gelukkig kan ik dat een klein beetje compenseren op de racefiets.
Lotte en Nicolette, lijdzaam hebben jullie moeten toekijken hoe het percentage mannen steeds verder toenam op D2 Oost. Jullie wisten echter het aantal borrels en andere activiteiten op peil te houden. **Vincent,** met een licht Amsterdams accent schuine moppen vertellen zorgde al snel voor een passende bijnaam. Daarnaast heb ik je ook leren kennen als een harde werker en iemand met een scherp analytisch vermogen. Dit heeft je ondanks wat tegenslagen een promotie opgeleverd om trots op te zijn. **Daisy,** in de voetsporen treden van Meike was natuurlijk niet makkelijk, maar door jouw enthousiasme, doorzettingsvermogen en aanstekelijke lach kreeg je alles voor elkaar. Ik heb genoten van onze qogea momenten, ski tripjes, borrels en uiteraard Miami. **Mike,** collega IBD onderzoeker, ik denk met veel plezier terug aan de vele bijeenkomsten die we samen bezocht hebben. Ik heb altijd erg moeten lachen om je acties en het feit dat je je niet door anderen laat beïnvloeden. Alleen jij bent in staat om midden in de kroeg een bowling bal tevoorschijn te halen, of beneden aan de berg uit te komen terwijl de rest aan het apres-skiën is boven op de berg. **Tim,** jij bent het bewijs dat topsport niet betekent dat je nooit meer naar de kroeg mag. Ik heb veel respect voor de manier waarop je je promotietraject hebt weten vorm te geven, tot snel in het Antonius! **Max,** buurman, ik hoefde mijn hoofd alleen maar naar rechts te draaien voor wat afleiding als ik mijn SPSS database zat was. Jimmy Kimmel, Taekwando, Youtube en niet te vergeten de dagelijkse imitatie van de frontcooklijn. Hoewel dit soms de productiviteit niet ten goede kwam, was het altijd fijn om jou als collega te hebben. **Mirjam,** wat grappig om na lange tijd jouw naam in de mail voorbij te zien komen voor een wetenschapsstage. Ik heb je leren kennen als enthousiast en leergierige collega, de surveillance studie laat ik dus met een gerust hart bij je achter.
Jorrit, ik heb altijd erg moeten lachen om jouw nuchtere, soms cynische blik op je projecten.
Susan, het feit dat je in 2 jaar tijd door je promotietraject heen bent gewalst en nu aan de opleiding gaat beginnen zegt echter genoeg over jouw capaciteiten.
Christine, Barrett en borrelen, die woorden komen bij jou in mij op. De overige collega's zullen begrijpen waarom ik moet lachen als ik dit hardop zeg. En last but not least **Wouter.** Soms een grote mond maar een klein hartje. Jij meer een doener en ik meer een denker maar uiteindelijk delen we toch opvallend vaak dezelfde mening. Het is altijd een plezier om met jou onder het

genot van een biertje of nog beter op de racefiets onze bezigheden te bespreken. Fijn dat je mijn paranimf wilt zijn!

Linda Wanders, dank voor je hulp bij de inclusie van de surveillance studie. Ik denk met plezier terug aan mijn bezoekjes aan het AMC voor overleg. Mooi dat onze gezamenlijke project geleid heeft tot een hoofdstuk in ons beider proefschrift.

Joyce en Willemijn, jullie wetenschappelijke stage heeft een belangrijke bijdrage geleverd aan enkele hoofdstukken van dit proefschrift. Gewapend met een OV-kaart en een lege database gingen jullie de ziekenhuizen af op zoek naar surveillance patiënten. Ik hoop dat ik jullie in ruil voor deze noeste arbeid iets heb kunnen bijbrengen over de opzet, analyse en het opschrijven van wetenschappelijk onderzoek.

Dr. H.H. Fidder, beste Herma. Dank voor het uitvoeren van alle 'smurfscopieen' en je kritische blik op enkele manuscripten uit dit proefschrift.

Endoscopie secretaresses, Cora, Marja, Eva, heel veel dank voor jullie hulp bij het steeds weer omgooien van het programma als er weer een 'smurfscopie' tussen gepropt moest worden.

Linda en Ada, samen de spil van de afdeling MDL. Heel veel dank voor jullie hulp bij allerlei vragen en regel dingen. Het was altijd fijn om bij jullie binnen te lopen voor een praatje.

En dan iedereen van het 'gevorderden groepje' van mijn fietsvereniging CS 030. Niets was fijner dan even wat stoom afblazen op de fiets door samen met jullie over de heuvelrug te blazen. Alle trainingen, weekendjes, vakantieweken in de Alpen waren een mooie afleiding en een goede manier om de batterij weer op te laden.

Bestuursgenootjes van het Orca bestuur 08-09, lieve butjes. Een gevoel van thuiskomen, anders kan ik onze samenkomsten de afgelopen jaren niet omschrijven. Altijd fijn om jullie weer te zien en te horen waar jullie mee bezig zijn. Dat er nog maar vele weekendjes en avonden samen mogen volgen. Op naar New York! (of een andere koningse bestemming)

Alle collega's en stafleden van de afdeling interne in het Antonius ziekenhuis, dank voor jullie begrip en prettige samenwerking tijdens mijn eerste stappen als dokter in de kliniek.

Ruud, Ineke en Emma, een betere schoonfamilie kan ik niet wensen. Dank voor jullie gastvrijheid, gezelligheid en interesse voor mijn bezigheden.

Lieve pap en mam, dank voor jullie grenzeloos vertrouwen en interesse voor alles wat ik doe. Pap, wat leuk dat we de afgelopen jaren hebben kunnen discussiëren over wetenschappelijk onderzoek en statistiek in het bijzonder. Fijn dat je mijn paranimf wilt zijn!

Marieke, Michael, Floris en Valerie, wat een feest om af en toe bij jullie langs te mogen. Dank voor jullie gastvrijheid, de deur stond altijd open voor een bord eten en een spelletje.

En dan tot slot mijn allerliefste Fleur. Jouw steun en liefde is grenzeloos. Dit proefschrift heeft vooral de laatste tijd heel wat van mijn tijd opgeslokt. Ik kijk ernaar uit om er vanaf nu weer samen op uit te trekken, beginnend met een reis naar Cuba. Ik hou van je!

CURRICULUM VITAE

Erik Mooiweer werd geboren op 2 juli 1985 in Nieuwegein. Na zijn Middelbare school periode op het Christelijk Gymnasium in Utrecht begon hij in 2003 aan de studie geneeskunde aan de Universiteit Utrecht. Hij onderbrak zijn studie in 2008 gedurende 1 jaar om als materiaal commissaris zitting te nemen in het bestuur van studenten roeivereniging Orca. In het laatste jaar van de studie geneeskunde startte hij met wetenschappelijk onderzoek op de afdeling maag-, darm-, leverziekten van het UMC Utrecht onder begeleiding van dr. B. Oldenburg. Dit onderzoek zette hij uiteindelijk voort in een promotietraject onder leiding van prof. dr. P.D. Siersema en dr. B. Oldenburg met als onderwerp surveillance voor colorectaal carcinoom en ontstekingsmarkers bij patiënten met inflammatoire darmziekten. In januari 2014 is hij begonnen met de opleiding tot maag-, darm-, leverarts, waarvan hij momenteel de vooropleiding interne geneeskunde doorloopt in het Antonius ziekenhuis Nieuwegein (opleider dr. A.B.M. Geers).