COLORECTAL CANCER AND IBD IN NORWAY

A RETROSPECTIVE ANALYSIS

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2 Abbreviations

5-ASA	5-aminosalicylic acid
APC	Anaphase-promoting complex
CD	Crohn`s disease
CRC	Colorectal cancer
DALM	Dysplasia associated lesion or mass
DNA	Deoxyribonucleic acid
IBD	Inflammatory Bowel Disease
ICD-9	International Statistical Classification of Disease and Related Health Problems 9th revision
MRR	Mortality rate ratio
P53	Tumor protein 53
PSC	Primary sclerosing cholangitis
SIR	Standardized incidence ratio
UC	Ulcerative colitis

3 Introduction

As a basis to understand the aim of the present study, a brief overview of the relevant aspects of inflammatory bowel disease (IBD) and colorectal cancer in IBD as well as important results of previous studies in the field will be given in this introduction.

3.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a group of disorders that causes inflammation of the intestine and sometimes also in organs external to the gut. The main forms of IBD are Crohn`s disease and ulcerative colitis. A third form, indeterminate colitis, comprises IBD cases in which a distinction between UC and CD cannot be made with certainty. Crohn's disease can affect any part of the alimentary tract, from mouth to anus, while ulcerative colitis is restricted to the large intestine, including the rectum. Crohns`s disease may show a variable extent of disease, with a defined border between affected and unaffected segments (skip lesions). Ulcerative colitis shows continuous inflammation, involving either the rectum only (proctitis), the rectum and the sigmoid (distal colitis), the distal colon up to the splenic flexure (left sided colitis), up to the hepatic flexure (substantial colitis) or including the coecum (pancolitis). Microscopically, Crohn`s disease may affect the whole bowel wall, whereas ulcerative colitis is limited to the inner lining of the gut, the epithelium.

The risk of developing IBD in Norway has been investigated in several prospective studies during the last two decades and has been found to be one of the highest in the world. The incidence of Crohns`s disease ranged from $5.3/10^5$ in western Norway [1] to $5.8/10^5$ in Northern as well as in South-Eastern Norway [2, 3]. The incidence of ulcerative colitis was $14.8/10^5$, $12.8/10^5$, and $13.6/10^5$, respectively [4-6]. Diagnosis is made by an endoscopic examination (colonoscopy) with biopsy. The etiology of IBD is complex, involving inherited susceptibility and environmental factors. Although not fully understood, the inflammation is

thought to result from inappropriate and ongoing activation of the mucosal immune system driven by the presence of normal gut microbes [7, 8].

The inflammation affecting the bowel wall causes symptoms ranging from abdominal pain and diarrhea in mild disease to blood in stool and weight loss in more severe cases. Onset of disease appears typically in young age, and once diagnosed, it is prevalent for the rest of the life, and may be invalidating due to complications. Many patients may, however, experience attenuating symptoms throughout course of disease, mainly as a consequence of treatment. Inflammation may subside spontaneously, but usually either medical or surgical treatment is necessary to get the symptoms under control and to prevent relapses.

3.2 Colorectal cancer in inflammatory bowel disease

Patients with inflammatory bowel disease are at increased risk of colorectal cancer (CRC) [9-12]. Due to a higher incidence of ulcerative colitis compared to Crohn`s disease, the risk of cancer has become more apparent in ulcerative colitis, but has also been recognized in Crohn`s disease with colonic involvement [13]. The mechanisms that lead to the initiation of neoplasia and the subsequent progression to cancer (carcinogenesis) are not fully understood. Some of the important hypothesis will be summarized in the following section. Cohort and case-control studies have unraveled associations between clinical and histological variables and cancer in IBD [14]. These risk factors will also be presented.

3.2.1 Carcinogenesis of CRC in IBD

Our understanding of the carcinogenesis in IBD has evolved during the past decades by learning from extensive research in sporadic colorectal cancer, which shows a much higher incidence rate. Progression to malignancy in sporadic cancer is thought to follow an adenoma-cancer sequence, where adenomas are visible precursor lesions of the cancer. Although there is a tendency to multiplicity among adenomas, most sporadic carcinomas appear as solitary

tumors and neoplastic changes are found only in one affected segment of the colon. One has observed that the genomic instability increases in line with growth of the adenomas, giving rise to a high risk of cancer in adenomas larger than 1 cm in diameter [15]. Genomic instability comprises chromosomal as well as microsatellite instability [16]. Chromosomal instability, as evidenced by gains and losses of chromosome arms, leads to the loss of function of key tumor suppressor genes, such as APC and p53. Microsatellite instability, detected by using PCR with a panel of microsatellite markers, involves loss of function of DNA base mismatch repair genes, resulting in DNA replication errors throughout the genome. Furthermore, epigenetic events, such as methylation, can contribute to altered gene expression in colon carcinogenesis.

The development of CRC in IBD shows many similarities with sporadic cancer, but also remarkable differences. Among these are lower age compared with sporadic CRC. The mean age at diagnosis of CRC in IBD is approximately 48 years [17, 18] as opposed to 70 years in sporadic CRC [19]. Similar to sporadic cancer, neoplastic progression in ulcerative colitis is thought to occur in a stepwise manner, but generally from flat mucosa and not from adenomas as for sporadic carcinomas. They are therefore considered to be more difficult to detect at endoscopy. The precursor lesions in CRC-IBD are defined by specific morphologic changes of the mucosal cells (dysplasia) and are being graded according to the severity of the changes: normal mucosa – indefinite for dysplasia – low and – high grade dysplasia – cancer [20]. Dysplasia is generally diagnosed in colorectal biopsies taken either randomly or directed by visible and suspicious lesions of the colon, in which dysplasia sometimes occurs - DALM (dysplasia associated lesion or mass). Neoplasia in IBD is often multifocal. Dysplasia is often present at several regions throughout the colon, and up to 27% percent of patients show two or three tumors simultaneously at the time of diagnosis [21, 22]. This phenomenon also relates to molecular changes related to neoplasia in IBD. Genomic instability is found both in

biopsies presenting dysplasia or cancer, but also in tissue that appears to be normal, i.e. without histological changes. The widespread molecular changes over a wider field than what is recognizable from structural damage, the so called “field effect”, has been postulated to characterize a wider area of genomic instability in the colon in IBD with a risk of malignant transformation [23-26]. On the one hand, there is evidence for the field effect to be as large as involving the entire colon [27], on the other hand several studies have shown that a considerable number of patients with CRC-IBD present with dysplasia contiguous but not distant from the cancer [28-30]. The interpretation of these findings is challenging and it is still unclear whether there are subgroups of CRC in IBD that do not follow the usual pattern of malignancy in IBD and whether there are several underlying molecular pathways of malignancy in IBD, that might modulate the field effect.

The theory of the field effect implies that the mechanisms that cause initiation and progression to malignancy in IBD probably are widely distributed throughout the colon, at least in a selection of IBD patients with neoplasia. To what extent this is connected to the distribution of inflammation is unclear. However, since inflammation is one of the main characteristics of IBD and typically involves large regions of the intestine, it has been proposed to play a central role in carcinogenesis related to IBD [31].

Two models for the development of malignancy have come into focus. One favors the assumption that chronic inflammation leads to telomere shortening. Telomers consists of repetitive DNA at the ends of the chromosomes, which protects the chromosomes from destruction. It shortens naturally with age but also by inflammation. Short telomers allow chromosome-ends to fuse together subsequently leading to chromosomal breaks, hence to gains and losses of chromosome arms [32]. The other model focuses on the relationship between inflammation and the production of reactive oxygen. Longstanding inflammation

might lead to oxidative stress, resulting in loss of DNA mismatch repair function or loss of other gene function and thus to genetic instability [33, 34].

Other clinical-histological features of CRC in IBD are quite similar to sporadic adenocarcinoma of the colorectum, although some differences have been observed. Some tumors in IBD may be entirely microscopic, without any abnormality of the mucosa at macroscopic inspection of the specimen [35]. A higher percentage of cancers in IBD occur in strictured segments of the colon [36, 37]. There seems to be a higher prevalence of mucinous carcinomas in IBD and more often advanced grade of malignancy compared with sporadic carcinomas [17, 28, 38].

3.2.2 Incidence and risk factors of CRC in ulcerative colitis

A population based study conducted in Sweden and published in 1990 reported a standardized incidence ratio (SIR, ratio of observed to expected cases) of 5.7 (95%CI: 4.6-7.0) for ulcerative colitis associated CRC. The risk of cancer was related to the *colonic extent* of disease. For patients with ulcerative proctitis the SIR was 1.7 (95%CI: 0.8-3.2), for those with left-sided colitis, 2.8 (95%CI: 1.6-4.4) and for those with substantial or pancolitis, 14.8 (95%CI: 11.4-18.9) [9]. The risk of cancer was also related to *age at diagnosis of ulcerative colitis*. For each increase in age group at diagnosis (<15 years, 15-29 years, 30-39 years, 40-49 years, 50-59 years and >60 years), the relative risk of CRC, adjusted for the extent of disease at diagnosis, decreased by about fifty percent (adjusted SIR 0.51; 95%CI: 0.46-0.56). *Duration of disease* seems to be another risk factor. A meta-analysis of 116 studies estimated a cumulative risk for any ulcerative colitis patient, irrespective of disease extent, to be 2% at 10 years, 8% at 20 years and 18% at 30 years of disease duration [11]. The overall prevalence of CRC in any patient with UC in this study was 3.7%, increasing to 5.4% for those with pancolitis [11]. In spite of the effect of duration of IBD on the risk of acquiring CRC, several patients were reported with development of CRC during the first decade after diagnosis of

IBD. Prior to the present study, systematically collected data addressing this problem had not been available. In the meantime, however, a study conducted in the Netherlands has been published, reporting as much as 22% of patients with IBD developing CRC before 8 years of disease in pancolitis or 15 years in left-sided colitis[39]. In that study no attempt was made to identify risk factors for early onset of CRC in IBD.

3.2.3 Incidence and risk factors of CRC in Crohn`s disease

As outlined above, Crohns`s disease may effect any part of the intestine, including the upper and lower gastrointestinal tract. Cancer in the small intestine related to Crohn`s disease has been observed, but the risk of adenocarcinoma is highest in patients with colonic inflammation only [10, 40]. The SIR for patients with Crohn`s disease with involvement of the colon only was 5.6 (95%CI: 2.1-12.2) in the aforementioned Swedish cohort [10]. Patients in whom Crohn`s disease was diagnosed before the age of 30 with any colonic involvement had a SIR of 9.5 (95%CI: 3.1-23.2) compared to a SIR of 1.6 (95%CI: 0.6-3.3) in patients older than 30 at diagnosis. The risk of CRC in Crohn`s disease was confirmed by a meta-analysis including 6 reports from the USA, Sweden, Denmark, Israel and Canada [12]. The pooled SIR for colon cancer in Crohn`s disease with colonic involvement was 2.5 (95%CI: 1.7-3.5).

The risk of cancer in ulcerative colitis and Crohn`s disease was directly compared in a study published in 1994 and found to be similar [41]. In this cohort, consisting of patients from England and Sweden, the relative risk for developing CRC, adjusted for age, gender, and years of follow up, was 19.2 (95%CI: 12.9-27.6) in ulcerative colitis and 18.2 (95%CI: 7.8-35.8) in Crohn`s disease. Although most cancers in Crohn`s disease have been found in segments of the colon with evidence of inflammation [42-44], in some cases the tumor has been diagnosed in regions of the intestine devoid of endoscopic or pathological signs of inflammation [36, 45-47]

3.2.4 Miscellaneous risk factors for CRC in IBD

Other important risk factors for CRC in IBD are the occurrence of CRC in relatives of the IBD patients, the severity of the colonic inflammation and the diagnosis of primary sclerosing cholangitis, which is an inflammation of the bile ducts due to an autoimmune reaction.

A population based cohort study of 19876 individuals with ulcerative colitis or Crohn`s disease, born between 1941 and 1955 examined the risk of CRC in IBD with and without a family history of CRC [48]. Patients who had a first degree relative with CRC had a relative risk of 2.0 (95%CI: 1.0-4.1) for the diagnosis of CRC compared to patients with no family history of CRC and the relative risk was higher for patients with a first degree relative who had been younger than the age of 50 at diagnosis of CRC. Interestingly, in that study, IBD patients with relatives who had also been diagnosed with IBD but not with CRC, had no increased risk of CRC compared to those IBD patients with no family history of IBD.

An early study of the course and prognosis of ulcerative colitis reported a higher risk of cancer in IBD patients with chronic continuous symptoms compared to chronic intermittent symptoms [49]. On the other hand, two subsequent studies analyzing the relationship between the frequency of clinical exacerbations and the risk of CRC failed to show an association [50, 51]. Recent publications, however, have given substantial support to the relationship between the severity of inflammation and the risk of cancer in IBD. A case control study from England in 2004 involved 68 cases and 136 matched controls and showed a correlation between neoplastic progression and an inflammation score that summarized the severity of inflammation in a single value covering the entire course of disease for each subject [52]. Adjusted for family history of CRC, medical treatment, smoking status, and concomitant primary sclerosing cholangitis, a 1-unit increase in this histological score increased the odds of CRC by a factor of 4.69 (95%CI: 2.10 -10.48, $p<0.001$). A subsequent cohort study from USA investigated the effect of changes in inflammation over time by using proportional

hazards analysis (Cox regression) of grade of histological inflammation coded as time changing covariate [53]. Of the 418 patients who all entered the colon cancer surveillance program without known dysplasia, 15 progressed to advanced neoplasia (five adenocarcinomas and ten high grade dysplasia) during follow-up. Adjusted for the frequency of endoscopy, a 1-unit increase in the histological inflammation score increased the risk of advanced neoplasia by a factor of 3.8 (95% CI: 1.7-8.6).

The risk of neoplasia in patients with IBD combined with PSC is controversial. Several studies have indicated that patients with IBD and PSC are at a higher risk of acquiring colorectal neoplasia [54-57], whereas larger studies from the Mayo clinic reported no risk [58] or only a borderline increased risk [59].

3.2.5 Protective factors for CRC in IBD

In addition to decreased symptom activity and reduced severity of inflammation, treatment of IBD with medical compounds such as Sulfasalzine or Mesalamine (5-ASA compounds) may have a protective effect on the neoplastic progression in IBD [60]. In a study involving 18969 patients with IBD in the UK General Practice Research Database, regular users of 5-ASA 0-12 and 12-24 months prior to the diagnosis of CRC had a significantly reduced risk of CRC compared with irregular users [61]. This study also showed a different risk reduction of Sulfasalazine compared to Mesalamine. Because Sulfasalazine inhibits the absorption of Folate [62] and Folate deficiency is correlated to a higher risk of sporadic neoplasia [63], it is possible, that the lack of cancer prevention of Sulfasalazine may be a result of reduced folate absorption [64]. The chemoprotective effect of mesalamine seems to be dose-dependent. A 5-ASA dose, equivalent to mesalamine of at least 1.2g/day provided the greatest risk reduction (72%-81%) [51, 65].

An important confounder of cancer protective factors in IBD might be the frequency of consultations with a clinician. Visiting a hospital doctor more than twice a year reduces the risk of cancer by 84% (p=0.006) [51].

3.2.6 Prognosis of CRC in IBD

Previous studies have shown conflicting results regarding the survival of CRC-IBD compared to CRC without IBD. A cohort study conducted in Great Britain in 1984 [18] and two case-control studies from the Mayo clinic, published in 1980 and 2006 reported similar survival in CRC-IBD compared to CRC without IBD [17, 66]. In contrast, two Danish studies in 2006/2007 showed poorer survival in CRC-IBD compared to CRC without IBD [67, 68]. While the studies from Great Britain and from the Mayo clinic were hospital based, the Danish studies were population based, by recruitment of the patients in a nationwide database between 1977 and 1999. Compared to 71000 patients with non-IBD CRC, the mortality rate ratio, adjusted for age and other important covariates, were 1.24 after one year and 1.17 after five years of follow up for 279 patients with ulcerative colitis-CRC and 1.82 and 1.57, respectively, for 100 patients with Crohn`s disease-CRC [67, 68]

3.2.7 The management of cancer risk in IBD

The question which is the best strategy to manage cancer risk in patients with IBD is controversial. Over the past 30 years, colonoscopic surveillance for dysplasia every second year from 8-10 years disease duration has been advocated [69], instead of preventive removal of the colon [70]. This strategy is based on the association of dysplasia and cancer. A systematic review of 10 prospective studies reported in 1994 that 42% of patients with high-grade dysplasia and 19% of patients with low-grade dysplasia had synchronous cancers at the time of the surgical removal of the colon (colectomy) [71], and that 16-29% of patients with untreated low-grade dysplasia progressed to DALM, high grade dysplasia or cancer. On the

other hand, screening for dysplasia has shown to face serious difficulties. As mentioned before, dysplasia is not visible by inspection and occurs in patches. It takes 33 or more random biopsies to have 90% confidence of finding dysplasia [70, 72]. Also the histological evaluation of the biopsies encounters uncertainty. There is a considerable variability in the diagnosis of low or high-grade dysplasia among pathologists (inter-observer variability) and also on a second reading of the same slide by the same pathologist (intra-observer variability) [20, 73, 74]. The subgroup of patients who develop cancer within eight years disease duration, that is, before guidelines recommend inclusion in surveillance programs might be erroneously excluded from surveillance [75]. This adds to the confusion regarding the recommendation of surveillance programs. A Cochrane review revealed recently, that there is no conclusive evidence that surveillance colonoscopy prolongs survival in patients with extensive colitis [70, 76].

3.3 Summary of introduction

In summary, patients with ulcerative colitis and with Crohn`s disease with colonic involvement are at increased risk of developing colorectal cancer. Risk factors include young age at onset of IBD, extensive disease and long disease duration, severity of inflammation, diagnosis of primary sclerosing cholangitis and genetic susceptibility. Some patients develop cancer early during the course of disease. The magnitude of this phenomenon is not yet thoroughly investigated and risk factors for early CRC in IBD have not been identified so far. Therapy with a 5-ASA compound probably reduces the risk of cancer. Compared to CRC in the general population, the prognosis of CRC in IBD is poor. Cancer screening strategies have not shown the desired efficacy in the prevention and early detection of cancer in IBD.
More knowledge about factors that influence carcinogenesis in IBD is needed, especially with regard to the possibility, that the etiology of CRC in IBD might be more heterogeneous than previously believed. In addition to patients with IBD and concomitant primary sclerosing

cholangitis, possible subgroups with increased risk of CRC in IBD have not been identified. It is also possible that disease behavior and risk factors are different between countries, which stress the importance of population based studies.

4 Aims of the study

1. To determine the variability of the colitis-CRC interval and to identify risk factors for early onset of CRC in IBD in a Norwegian population based cohort.
2. To identify new subgroups of CRC in IBD by analyzing the histological variability of CRC in IBD and possible associations with clinical parameters.
3. To analyze the effect of clinical and histological factors on the prognosis of CRC in IBD.

5 List of Papers

5.1 Paper I

Brackmann, S.A., Andersen, S.N., Aamodt, G., Langmark, F., Clausen, O.P.F., Aadland, E., Fausa, O., Rydning, A., Vatn, M.H., *Relationship between clinical parameters and the colitis-colorectal cancer interval in a cohort of patients with colorectal cancer in inflammatory bowel disease.* Scandinavian Journal of Gastroenterology, 2008. **Apr 1**: p. 1-10. [Epub ahead of print].

5.2 Paper II

Brackmann, S.A., Andersen, S.N., Aamodt, G., Roald, B., Langmark, F., Clausen, O.P.F., Aadland, E., Fausa, O., Rydning, A., Vatn, M.H., *Two distinct groups of colorectal cancer in inflammatory bowel disease.* Inflammatory bowel diseases, 2008. **Jul 10**: [Epub ahead of print].

5.3 Paper III

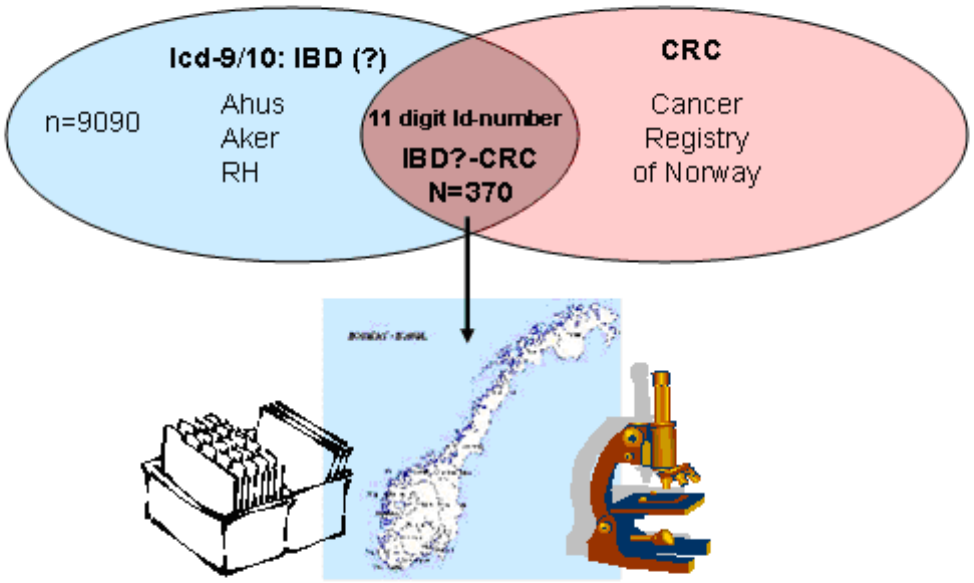
Stephan Brackmann, Geir Aamodt, Solveig Norheim Andersen, Borghild Roald, Frøydis Langmark, Ole PF Clausen, Erling Aadland, Olav Fausa, Andreas Rydning, Morten H.Vatn., *Widespread but not localized neoplasia in inflammatory bowel disease worsens the prognosis of colorectal cancer.* Submitted

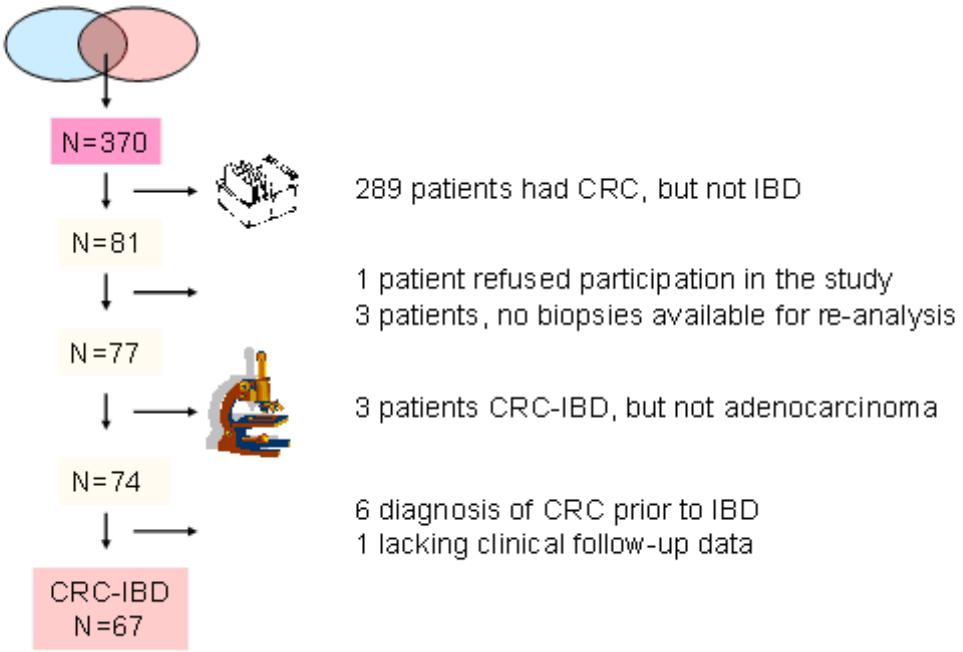
6 Methodological considerations

6.1 Recruitment of the patients with CRC-IBD

All patients who had been electronically recorded with the diagnosis of Crohn`s disease (International Statistical Classification of Disease and Related Health Problems 9th revision (ICD-9); 555 and 10th revision (ICD-10); K50), or with ulcerative colitis (ICD-9: 556, ICD-10: K51) or non-infectious colitis (ICD-9: 153, ICD-10: K52) at Rikshospitalet University Hospital in the period 28.02.1973 – 30.04.2005 (n=3285), at Akershus University Hospital in the period 12.12.1985-09.02.2004 (n=4795) as well as patients from the prospectively recorded IBD cohort from Aker University Hospital (1987-2004, n=1010) were matched against the CRC files (ICD-9: 153, ICD-10: C18) at the Cancer Registry of Norway.
Thus, out of 9090 patients with a possible diagnosis of IBD, 370 patients with the diagnosis of cancer in the colorectum were identified. Five patients who had been diagnosed with CRC in IBD at Rikshospitalet University Hospital prior to 1973 were also included.
By review of clinical data and histological re-analysis of the biological material, 289 patients were confirmed of having CRC but showed no evidence of IBD. The majority of these patients had been diagnosed with diarrhea secondary to adjuvant therapy of sporadic cancer and had thus been coded with the “non-infectious colitis” ICD-codes. Of the remaining 81 tentative study cases, several patients had to be excluded: One patient refused participation in the study, in three patients biopsies for re-analysis were no longer available, one patient had carcinoid cancer in IBD, one neuroendocrine differentiated carcinoma in IBD and one squamous cell carcinoma of the anus, six patients were diagnosed with adenocarcinoma in the colorectum prior to the diagnosis of IBD and one patient was completely lacking any clinical follow-up data. (figure1). Consequently, 67 patients with the diagnosis of adenocarcinoma in

Figure 1) Recruitment of the patients with colorectal cancer in inflammatory bowel disease





the colorectum (CRC) in either ulcerative colitis (UC) or Crohn`s disease (CD) were included in the cohort. There were no patients with indeterminate colitis and CRC.

6.2 Data collection

Patient demographics, endoscopic findings, pathology reports and clinical data regarding the course of disease and treatment were reviewed and paraffin-embedded material from colectomy or endoscopy was re-analyzed. In case of incomplete files at the recruiting hospitals, patient journals were traced throughout health institutions in Norway and biopsies were collected from other hospitals when necessary. When original histological slides were not available, new slides were cut from the existing paraffin-blocks. All slides were evaluated by one experienced pathologist in a blinded fashion. When the re-evaluation deviated from the original pathology report, a consensus was achieved by consulting a third experienced pathologist.

6.3 Statistics

Statistical analyses were performed by using SPSS 14.0. Groups were compared by using independent T-tests or one-way ANOVA for continuous outcome variables and Pearson Chi-Square or Fisher`s Exact test for categorical outcome variables. Bonferroni correction was used for comparisons of groups with more than two categories. A dependent variable was explained by a set of independent factors by using linear regression analysis. Significant ($p<0.05$) and close to statistical significant factors ($p<0.1$) were stepwise included into a multiple linear regression model. In multiple logistic regression analysis, forward variable selection procedure was used to find the model that best predicted the factor of interest. For survival analysis, Cox regression was used to compute hazard ratios for death (mortality rate ratio) in CRC-IBD compared to all CRC in the general population, adjusting for cofactors.

6.4 Ethics

The study protocol was approved by the Regional Committee for Medical and Health Research Ethics. Personal data was handled according to the license of the Data Inspectorate. Patients were requested for written informed consent. In case of a deceased patient data were extracted by the permission of the Department of Health and Social Affairs.

7 Summary of the results

7.1 Paper I

Relationship between clinical parameters and the colitis-colorectal cancer interval in a cohort of patients with colorectal cancer in inflammatory bowel disease

The study investigated the variability of the colitis-CRC interval and possible clinical risk factors for early onset of CRC in IBD.

The median time from diagnosis of IBD to CRC was 17 years. Twelve percent of the patients developed CRC within 10 years from onset of IBD symptoms and 21% within 10 years after the diagnosis being confirmed. High age at onset of IBD symptoms and a high percentage of the colitis-CRC interval with active symptoms was associated to the development of CRC early in the course of IBD. High age at onset of IBD was also associated with advanced stage of cancer at diagnosis of CRC. Patients with PSC were significantly younger at onset of IBD symptoms, but the colitis-CRC interval was similar to IBD without PSC. Other potential clinical risk factors, such as the family history of CRC or IBD, and drug treatment, were not associated to the duration of the colitis-CRC interval.

In summary, 21 % of the patients in the cohort developed cancer before screening with colonoscopy usually is recommended. High age at onset of IBD seems to be related to early CRC in IBD.

7.2 Paper II

Two distinct groups of colorectal cancer in inflammatory bowel disease

The study investigated the histological variability of CRC in IBD and associations with clinical parameters.

Seventy-seven adenocarcinomas were diagnosed among the 67 patients. Fifty-nine patients (88%) had one - and eight patients (12%) had multiple cancers in the colorectum. Dysplasia was found distant to the tumor in 43 of 60 (75%) patients. In 17 patients (25%), dysplasia was only presented contiguous to the cancer.

The presence of multiple cancers or distant dysplasia in the colorectum (widespread neoplasia in IBD) was associated to young age at onset of IBD, a long duration of the colitis-CRC interval and the presence of active inflammation at time of the cancer diagnosis. Patients with no evidence of neoplasia apart from the tumor (localized Neoplasia in IBD) showed a higher age at onset of IBD and a wide variation of the colitis-CRC interval. However, the average duration of time from onset of IBD to diagnosis of CRC was significantly shorter compared to patients with widespread neoplasia in IBD. In fact, all patients diagnosed with cancer within ten years from onset of symptoms had localized neoplasia in IBD. By multiple logistic regression analysis, age at onset of IBD symptoms was the strongest predictor for distant dysplasia at time of cancer diagnosis.

In summary, widespread and multifocal neoplasia occurs in the majority of cases with CRC in IBD and is associated to early onset of IBD. However, localized neoplasia may occur in about a quarter of the patients and shows an association to late onset of IBD. The two groups may represent different pathogenetic entities of neoplasia in IBD.

7.3 Paper III

Widespread but not localized neoplasia in inflammatory bowel disease worsens the prognosis of colorectal cancer

In this study we compared survival of CRC in IBD with CRC in the general population (all-CRC), adjusting for possible general confounders and strictly IBD related cofactors including new variables, such as type of CRC in IBD.

Adjusted for cofactors, prognosis of CRC-IBD was poorer compared to all-CRC (mortality rate ratio (MRR) 3.71, 95%CI: 2.54-5.42, $p<0.001$). Prognosis of widespread neoplasia-IBD was poorer compared to all-CRC (MRR 4.27, 95%CI: 2.83-6.44, $p<0.001$) and compared to localized neoplasia-IBD (MRR 3.58, 95%CI: 0.87-14.72, $p=0.076$). Survival was not significantly different between localized neoplasia-IBD and all-CRC ($p=0.132$). The mean age at CRC diagnosis was 43 years in widespread, 52 years in localized neoplasia-IBD, and 70 years in all-CRC. Patients with CRC-IBD younger than 45 years at cancer diagnosis had a shorter survival than those above 45 years; mortality rate ratio 3.9 (95%CI: 2.2-6.9) versus 3.2 (95%CI: 1.9-5.5), respectively.

In summary, CRC in IBD showed poorer prognosis compared to all-CRC. The unfavorable effect of IBD on survival after diagnosis of CRC was more pronounced in patients below 45 years of age and in patients with widespread neoplasia in IBD. Low age at diagnosis may be a confounder for widespread neoplasia in IBD. The diagnosis of the topographical distribution of dysplasia seems to be an important prognostic factor in patients with CRC in IBD.

8 Discussion

In this retrospective analysis we investigated colorectal cancer in IBD in Norway. The aim was to gain more knowledge about factors that influence carcinogenesis in IBD, especially with regard to the possibility that the etiology of CRC in IBD might be far more heterogeneous than so far understood. In the following section, the main results of our investigations will be reviewed in the context of the findings of other relevant studies and the possible contribution of our results to the understanding of CRC in IBD will be discussed.

8.1 The colitis-CRC interval

Twelve percent of the patients developed CRC within 10 years from onset of IBD symptoms and 21% within 10 years of having the diagnosis confirmed. International guidelines suggest to start surveillance in IBD after 8-10 years from onset of disease in patients with pancolitis and after 15 years in left sided-colitis [69, 77]. Hence, 21% of the patients with CRC in IBD in our cohort were diagnosed with malignancy before the start of surveillance usually is recommended. Similar figures have been reported by colleagues from the Netherlands investigating 149 patients, diagnosed with CRC in IBD between 1990 and 2006 [39]. There seems to be a high frequency of early CRC in IBD and it appears to be a worthwhile attempt to identify factors associated with this phenotype.

We found an inverse relationship between age at onset of IBD and the duration of the colitis-CRC interval. This association is known from two previous reports but has been criticized of being secondary to a selection bias, taking into consideration, that older people are at higher risk of dying before CRC in IBD may have developed [78],[79]. In contrast to this interpretation, we found a higher frequency of advanced cancer stages in late onset IBD, indicating the possibility of a more aggressive type of carcinogenesis in these patients. To our knowledge, we were the first to investigate specifically the effect of other clinical variables

and the duration of the colitis-CRC interval, but found no predictors of early CRC in IBD, except the duration of active symptoms measured as percentage of the colitis-CRC interval. Interestingly, medical treatment, which is advocated as being protective for CRC in IBD, did not show a prolonged duration of disease until cancer, and vice versa, primary sclerosing cholangitis, which is presumed to be a aggressive risk factor for CRC in IBD, did not show an association to the development of cancer early during course of disease. Clinical factors alone could not sufficiently explain early CRC in IBD and it seemed reasonable to continue the search for new factors by analyzing histological characteristics in the cohort.

8.2 Two groups of CRC in IBD

A total of 915 paraffin blocks from 67 patients with CRC in IBD were reviewed. We found widespread, multifocal neoplasia in the majority, but localized neoplasia in about a quarter of the patients. These results can in part be interpreted in the light of earlier studies. Dysplasia being absent apart from the tumor in 25% of CRC-IBD has been recognized previously in several studies [28-30]. These studies were conducted when dysplasia as a marker for malignancy was relatively new and its predictive value for concurrent cancer the focus of investigation. The interpretation of the results addressed the question whether dysplasia was a predictive factor for cancer in IBD or not, and did not include the concept of different extension of dysplasia representing specific entities of CRC in IBD. As summarized by Ransohoff et al [30]: “The results suggest that failure to detect dysplasia may not exclude the presence of concurrent carcinoma”. The association between clinical factors and the topographical distribution of dysplasia was not analyzed in their studies.

In our cohort, widespread neoplasia in IBD was related to young age at onset and long duration of disease, and furthermore, to active inflammation at time of cancer diagnosis. Thus, this type of CRC in IBD seems to follow the traditional model of malignant development in IBD. Carcinogenesis in these patients appears to be initiated at young age and promoted

during a considerable time of disease in a wide field of the colon, possibly by IBD related factors, as for example inflammation. This would explain an increasing risk of cancer with duration of IBD and why this group rarely if ever develops malignancy within the first eight to ten years of disease.

We found localized neoplasia in IBD in about a quarter of the patients. These patients appear not to follow the traditional risk pattern of malignancy in IBD, as they showed late onset of IBD and in median, a relatively short colitis-CRC interval, including all patients with a diagnosis of CRC before the start of surveillance usually is recommended. We speculate that in localized neoplasia in IBD, initiation of the cancer may take place unrelated to IBD and that malignant changes, similar to sporadic CRC, accumulate over time in a small field of the colon before the onset of IBD. With onset of disease, IBD related immunogenic factors may accelerate the age related carcinogenesis in these patients, resulting in dysplasia and subsequently cancer at a single location before long-standing inflammation or other IBD related factors may give rise to neoplasia in a large field of the colon. Consequently, patients with advanced malignant changes prior to onset of IBD may progress to cancer within a shorter time than patients with an earlier stage of carcinogenesis at onset of disease. This model would explain the relatively wide variability of the colitis-CRC interval in patients with localized neoplasia in IBD and the fact that some are being diagnosed with CRC shortly after onset of IBD, or even before the onset of IBD symptoms, as shown in several cohorts, including our own.

8.3 Survival

Compared to CRC in the general population, we found poorer survival of CRC in IBD, thus confirming the results of a Danish cohort [67, 68]. The fact that the Danish and our cohort were population based, might be the reason for the diverging results compared to earlier case-control studies [17, 66]. Earlier studies have also been limited by the fact that IBD related

factors were lacking in the survival analysis. In the present study, we were able to include several new and relevant cofactors.

Young age at diagnosis of CRC and widespread neoplasia in IBD seemed to be related to poor prognosis in our cohort. We concluded, that age at diagnosis of CRC in IBD may be a confounder for the extension of neoplasia in the colon and suggest, that widespread neoplasia in the colon explains the poor prognosis of patients with CRC in IBD. Localized neoplasia in IBD showed no statistically significant difference in survival probabilities compared to all CRC in Norway. This might support the hypothesis that localized neoplasia in IBD might be related to sporadic CRC in the population. Other important cofactors, including IBD specific variables, as for example medical treatment, the number of colonoscopies during course of disease or the diagnosis of primary sclerosing cholangitis, seemed not to modulate prognosis in our cohort.

The diagnosis of the topographical distribution of dysplasia in the colon seems to be important for the assessment of prognosis in patients with CRC in IBD.

8.4 Limitations and strength of the study

The strength of our study was the use of the nationwide Cancer Registry of Norway as well as the thorough diagnostic work-up of the patients with CRC in IBD. Thus, we were able to reduce the selection bias to a minimum and include new factors relevant for IBD into analysis. A limitation of the study may be the lack of information about the causes of death. Whether death was directly related to CRC, for example to complications after surgery or to other factors, remains unknown. Another weakness of the study may be the limited number of patients with CRC in IBD, in spite of the fact that the size of our cohort is large compared to other studies. The close to normal distribution of the factor “duration of the colitis-CRC interval” may indicate a high representativity of the cohort. However, we were not able to

differentiate type of IBD in the analyses, i.e. ulcerative colitis versus Crohn`s disease, because the number of cases were too small.

8.5 Conclusions

In this retrospective analysis, we investigated the variability of clinical and histological factors in a population based Norwegian cohort of CRC in IBD. We also analyzed survival probabilities adjusted for cofactors which have so far not been subject of investigation in such patients. The main results of the studies can be summarized chronologically as follows:

- Twelve percent of the patients experienced CRC in less than 10 years after the onset of IBD symptoms and 21% within 10 years after having the diagnosis confirmed.
- High age at onset of IBD symptoms was associated to the development of CRC early during course of disease.
- In 75% of the patients, we found multiple cancers and/or dysplasia distant to the tumor (widespread neoplasia in IBD). It was associated with young age at onset of IBD, a relatively long colitis-CRC interval and active inflammation at cancer diagnosis. In 25% of the patients, neoplasia was only presented contiguous to the tumor (localized neoplasia in IBD). It was associated with a relatively high age at onset of IBD and a short colitis-CRC interval, including all patients who were diagnosed with cancer within 10 years from onset of symptoms.
- Compared to all CRC in the background population, young age at diagnosis of CRC in IBD and widespread neoplasia in IBD showed poor prognosis.

Based on the present results, not only the diagnosis of dysplasia but also the topographical distribution of dysplasia in the colon may be important to recognize during surveillance of IBD. Therefore, a high number of random biopsies and techniques with improved diagnostic yield, as chromoendoscopy, may be considered for cancer screening. One should also be

aware of the possibility, that patients with late onset of IBD may develop malignancy, unrelated to disease duration. By use of a combination of histological, clinical and demographic risk factors, one may reach at an early estimation of prognosis and relevant time of surgery, and as a consequence, a prolonged survival, in these patients. Future studies will improve our understanding of the disease mechanisms behind the divergent subgroups of patients with CRC in IBD.

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10 Original Papers