Ulcerative colitis
Colorectal cancer risk and surveillance in an unselected population

Jan Lindberg
When I heard how much there is,  
I thought that it would be easier to care a little  
than to not give a shit at all.

furu, näsdukar, strumpor m.m.

När jag fick höra hur mycket som finns,  
tyckte jag att det verkade lättare att bry sig lite  
än att skita i allt.

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ABBREVIATIONS USED IN THIS THESIS

CD  Crohn’s disease
CE  Chromoendoscopy
CI  Confidence interval
CK  Cytokeratin
CRC  Colorectal cancer
DALM  Dysplasia associated lesion or mass
DNA  Deoxyribonucleic acid
ERCP  Endoscopic retrograde cholangiopancreatography
HGD  High-grade dysplasia
IBD  Inflammatory bowel disease
ID  Indefinite for dysplasia
IPAA  Pouch-anal anastomosis
IRA  Ileo-rectal anastomosis
LC  Left-sided colitis
LGD  Low-grade dysplasia
MRCP  Magnetic resonance cholangiopancreatography
PPC  Panproctocolectomy
PSC  Primary sclerosing cholangitis
QOL  Quality of life
SIR  Standard incidence ratio
SMR  Standard mortality ratio
TC  Total colitis
TRD  Therapy resistant disease
UC  Ulcerative colitis
ABSTRACT

Ulcerative colitis
Colorectal cancer risk and surveillance in an unselected population
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Ulcerative colitis is a chronic inflammatory disease that mainly affects the colon and rectum. Onset of disease is most common between the ages of 15-35 years. There is an observed increased risk of colorectal cancer associated with the disease. The risk is often described to be 2% after 10 years, 8% after 20 years and 18% after 30 years disease.

Since 1977, all known patients with ulcerative colitis in the catchment area of Örnsköldsvik Hospital have been invited to attend a colonoscopic surveillance programme. At endpoint of the studies included in this thesis there were 214 patients that had attended the surveillance programme. The aims of these studies have been to evaluate the efficiency of the surveillance programme, analyse the impact of findings of DNA aneuploidy, and determine the outcome for patients that underwent limited resections instead of complete proctocolectomy. Further, we have studied the long-term outcome for patients who had an early onset of disease and analysed the expression of cytokeratin 7 and 20 in respect to findings of dysplasia, DNA aneuploidy and colorectal cancer.

At the end of the studies the prevalence for ulcerative colitis was 261/100 000 and the incidence rate was 7.6/100 000/year. During the period 1977-2005, four patients died of ulcerative colitis. Nine colorectal cancers were diagnosed in eight patients, one of whom died of the cancer. Fifty-two patients had findings of dysplasia and five of these patients developed colorectal cancer. In the subgroup of patients studied (N= 147) for DNA aneuploidy, 20 were found to have specimens with DNA aneuploidy on at least one occasion.

The investigation of the outcome for the patients that underwent limited resections of the colon or rectum showed that none of the patients under surveillance died of colorectal cancer or metachronous cancer in their remaining colon or rectum. A separate study concerning early onset of ulcerative colitis revealed no particular increased risk of colorectal cancer in this cohort but a fairly high incidence of primary sclerosing cholangitis was seen. In the analyses of cytokeratins it was found that 7 out of 10 patients with low-grade dysplasia and 3 of 6 with high-grade dysplasia were positive for CK7. Our results indicate a possible relationship between the expression of CK7 and CK20 and neoplastic development of colorectal mucosa in patients with ulcerative colitis. The studies on which this thesis is based, were performed on a relatively small number of patients, however the time of observation was long and, most importantly, the patients were from a well defined catchment area.

We conclude that the surveillance programme has been efficient in minimising the risk of lethal colorectal cancer. Analysing DNA ploidy helps to target the patients that need more attention but the method cannot stand alone. Our study on cytokeratins points to a relationship between dysplasia and CK7 but the results are preliminary and further studies needs to be done.

We have shown that it is safe to do a limited colorectal resection in respect to lethal colorectal cancer. Early onset of ulcerative colitis as a risk factor for colorectal cancer was not found in the group we have studied, which could be due to effective surveillance and/or medication. A fairly high rate of surgery in this group may also have contributed. The most important variable in the beneficial outcome regarding lethal colorectal cancer in these studies is, in our opinion, the outstanding compliance of the patients to the colonoscopic surveillance programme.

Key words: Ulcerative colitis, colonoscopic surveillance, colorectal cancer, dysplasia, DNA aneuploidy, cytokeratin, colorectal resection, early onset
POPULÄRVETENSKAPLIG SAMMANFATTNING


Sedan 1977 har alla kända patienter med ulceröös kolit inom Örnsköldsviks sjukhus upptagningsområde inbjudits att delta i ett kontrollprogram med regelbundna koloskopier. När studierna i denna avhandling slutfördes hade 214 patienter deltagit i kontrollprogrammet.

Syftet med studierna har varit att utvärdera kontrollprogrammet avseende dess effektivitet, analysera betydelsen av avvikelse i DNA och bestämma utfallet för patienter som opererats med begränsade resektioner i stället för kompletter borttagande av grov- och ändtarm som vanligen rekommenderas vid fynd av cancer eller cellförändringar (dysplasi). Vidare har vi studerat hur det, på lång sikt, har gått för de patienter som insjuknade i ung ålder (18 år och yngre) samt analyserat uttrycket av cellvägpotenser i cytkoteratinen cytkoteratin 7 och 20 (CK7 och CK20) i relation till fynd av dysplasi, DNA avvikelse och cancer i grov- och ändtarm.

Vid slutet av studietiden fanns 261 patienter /100 000 invånare och takten av insjuknande hade under åren varit 7,6/100 000/år. Under perioden 1977-2005 avled fyra patienter pga. av ulceröös kolit. Nio stycken cancar i grov- och ändtarm hade hittats hos åtta patienter, av vilken en avled till fält hära. Femtiotvå patienter hade haft dysplasi av vilka fem utvecklade cancer. I den gruppen av patienter (N=147) som studerades med avseende på DNA-avvikelse återfanns sådan avvikelse vid minst ett tillfälle hos 20 patienter.

Analyserna av hur det har gått för de patienter som opererats med begränsade resektioner i stället för kompletter borttagande av grov- och ändtarm visade att ingen patient som varit under koloskopisk kontroll avlidit till följd av cancer i grov- eller ändtarm.

I den separata studie som gjordes avseende de patienter som insjuknade före 19 års ålder fann vi ingen ökad risk för tarmcancer, men en relativt hög risk för en gallgängssjukdom vid namn primär skleroserande cholangit.

Vid analyserna av cytkoteratiner såg man att 7 av 10 patienter med läggradig dysplasi och 3/6 patienter med höggradig dysplasi var positiva för CK7. Våra resultat indikerar att det finns ett möjligt samband mellan uttryck av CK7 och CK20 och utveckling av dysplasi i grov- och ändtarm hos patienter med ulceröös kolit.

Studierna som denna avhandling bygger på är utförda på ett relativt litet antal patienter men observationstiden är lång och, viktigast av allt, patienterna kommer från ett väl definierat upptagningsområde.

Sammanfattningsvis drar vi slutsatserna att kontrollprogrammet med regelbundna koloskopier har varit effektivt vad gäller att minska dödsfallet i grov- och ändtarmscancer. Analyser av DNA är användbara vad gäller att identifiera patienter som kan vara i behov av större uppmärksamhet och meden man inte användas som ensam markör för potentiell dysplasi eller cancer. Studierna av cytkoteratiner pekar mot ett samband mellan dysplasi och CK7 men resultaten är preliminära och ytterligare studier är nödvändiga.

Vi har visat att det, gällande dödlig grov- och ändtarmcancer, är säkert att utföra en begränsad tarmresektion.

Vi har inte funnit någon ökad risk för grov- och ändtarmcancer hos patienter med tidig sjukdomsdebut av ulceröös kolit, detta kan bero på ett effektivt kontrollprogram och/eller medicinering. En tämligen hög frekvens av tarmoperationer i denna grupp av patienter kan också vara en bidragande förklaring.

Den viktigaste anledningen till få fall av dödlig grov- och ändtarmcancer i dessa studier är, enligt vår åsikt, den utomordentliga uppslutningen från patienterna till det koloskopiska kontrollprogrammet.
This thesis is based on the following papers, referred to in the text by their roman numerals:

I. Lindberg J, Stenling R, Palmqvist R, Rutegård J. 
Efficiency of colorectal cancer surveillance in patients with ulcerative colitis: 26 years’ experience in a patient cohort from a defined catchment area. 

II. Lindberg J, Stenling R, Rutegård J. 
DNA aneuploidy as a marker of premalignancy in surveillance of patients with ulcerative colitis. 

III. Lindberg J, Stenling R, Palmqvist R, Rutegård J. 
Surgery for neoplastic changes in ulcerative colitis-can limited resection be justified? Outcome for patients who underwent limited surgery. 

IV. Lindberg J, Stenling R, Palmqvist R, Rutegård J. 
Early onset of ulcerative colitis. Long term follow up with special reference to colorectal cancer and primary sclerosing cholangitis. 
Submitted for publication.

V. Stenling R, Lindberg J, Rutegård J, Palmqvist R. 
Altered expression of CK7 and CK20 in pre-neoplastic and neoplastic lesions in ulcerative colitis. 
APMIS. Accepted for publication.

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1. INTRODUCTION

Ulcerative colitis (UC) is an inflammatory disease targeting the large intestine. The disease is chronic but symptoms of urgency diarrhea, with or without mucus and/or blood and sometimes abdominal pain and tenesmus are intermittently exacerbating and remitting. The onset can be almost imperceptible but is in rare cases fulminant, requiring immediate surgery. Asymptomatic periods vary from months to years. The extent of inflammation can be restricted to the rectum (proctitis) or the rectum and sigmoid colon (proctosigmoiditis). Distal inflammation is more often associated with symptoms of urgency. Inflammation can also be extended to the splenic flexure (left-sided colitis), the hepatic flexure (extensive colitis) or involve the entire colon and rectum (total colitis). The seriousness of the disease is often dependent upon the extent of inflammation whereas severity is dependent upon the intensity of the inflammation. A limited but intensely inflamed section of the colorectum can be of utmost difficulty for the patient.

Extra-intestinal manifestations, such as inflammatory reactions in the skin, joints, eyes and hepatobiliary system are sometimes present. More recently described involvements are pulmonary diseases, thromboembolic events, osteopenia and osteoporosis\textsuperscript{1,2}.

The incidence of UC is calculated to be 1-25 per 100 000 per year and the disease affects people of all ages\textsuperscript{3-5}. Onset of disease in the first decade of life is unusual but there is a steep increase in incidence during puberty and the following adolescence and young adulthood\textsuperscript{6-8}. Prevalence has been calculated to be 234-303 per 100 000\textsuperscript{9,10}.

The etiology of the disease is yet to be revealed but the pathogenesis is believed to be multifactorial. An observed tendency of UC to cluster to families has implied that genetic components are involved in the development of the disease.

Five to twenty percent of the patients with UC or Crohn’s disease (CD) have a family history of inflammatory bowel disease (IBD)\textsuperscript{11}. In one study, the prevalence of UC was five times the expected in offspring of patients with UC\textsuperscript{12}. There is no evidence that any specific environmental factor has a role in the development of UC but a recent co-twin control study observed an association between recurrent gastrointestinal infections up to age 20 and UC\textsuperscript{13}. The most consistent exogenous factor in different studies is smoking, which shows an inverse association with UC. That is, cigarette smoking seems to have a protective effect against the development of UC and cessation of smoking is associated
with a risk of developing UC\textsuperscript{13,14}. It has been proposed that the association between UC and respiratory diseases could be a factor that explains the inverse association between UC and smoking\textsuperscript{15}. The etiopathogenesis of UC is today not elucidated but consensus is that the disease is caused by aberrant host response to yet unknown environmental antigens in genetically susceptible individuals with a perturbed immune regulation. The diagnosis of UC is based on the presented symptoms in combination with typical macroscopic findings in the colorectal mucosa on colonoscopy supported by histological findings on mucosal biopsies. Differential diagnoses such as entero-colitis caused by infection or antibiotics must be excluded. Other forms of non-infectious colitis such as vasculitis, microscopic colitis and Crohn’s disease can be more difficult to differentiate from UC. Up to ten percent of the patients with colonic disease have symptoms and signs suggestive, but not diagnostic, of Crohn’s disease. These patients are diagnosed as having “indeterminate colitis” and are often medically treated as the patients with UC. One to three percent of the patients with UC and Crohn’s disease will receive a change in diagnosis during the course of their disease\textsuperscript{16}. 
2. BACKGROUND TO THE PRESENT STUDIES

2.1 Mortality

Ulcerative colitis is a profoundly studied disease. One of the reasons for this is early reports of high mortality. Edwards and Truelove reported in a study covering 624 patients during 1938-1962 that 8 percent of the patients presenting their first attack of disease were dead within one year from referral. A long-term mortality rate of 30 percent was found if the initial attack was classified as severe\textsuperscript{17}. A mortality risk of 1.7 times that of the general population was reported by Gyde et al in their series of 676 patients during the time period 1940-1976\textsuperscript{18}. In a study analysing the long-time prognosis of 396 patients with onset of disease at age 14 or younger in the years 1919 through 1965 by Devroede et al, a death rate of 20 per cent per decade was found\textsuperscript{19}.

Fortunately, these depressing figures of mortality are not seen today. The use of systemic corticosteroids and cyclosporine in treatment of acute exacerbation and sulfasalazine or 5-aminosalicylic acid in maintenance therapy has been shown to reduce relapse rate and the severity of the relapses\textsuperscript{20-22}. The accumulated knowledge of pre-, per-, and postoperative management as well as the timing of surgery have also contributed to the decline in mortality rates\textsuperscript{23-26}.

A population-based study from Broström et al including 1274 patients diagnosed 1955-1979 and followed retrospectively from diagnosis up to 25 years showed a tendency toward better survival among the patients diagnosed 1970-1979 compared to those diagnosed 1955-1969\textsuperscript{27}. Winther et al showed in a study based on 1160 patients that a sub-group of patients, >50 years of age and with extensive colitis at diagnosis, had increased mortality within the first 2 years after diagnosis, owing to colitis-associated postoperative complications and comorbidity. Apart from this, no significant excess in mortality was found overall\textsuperscript{28}. Similar results in long-term survival were reported by Jess et al in a study from Olmsted County in Minnesota\textsuperscript{29}. They found that overall survival was similar to that expected in the US White population. The slight increase in mortality that has been seen during the first years of disease is addressed to severe fulminant attacks with perforation and bleeding as well as cardiovascular and infectious postoperative complications\textsuperscript{28,15}.

Even if mortality is not higher than the expected in the long-term studies cited above, there are UC related causes of death other than colorectal cancer (CRC).
In the study by Ekbom at al, there was an increase in deaths caused by non-alcoholic liver diseases with a standard mortality ratio (SMR) of 3.9 (95% confidence interval (CI) 2.0-7.0), asthma (SMR 3.3; 95% CI 1.2-7.3) and bronchitis and emphysema (SMR 2.6; 95% CI 1.3-4.6)\textsuperscript{15}. Jess et al found that death caused by gastrointestinal disorders including liver diseases was increased, but not significantly and that, on the other hand, cardiovascular death was reduced (SMR 0.6; 95% CI 0.4-0.9)\textsuperscript{32}. A European-wide population-based cohort study showed a significant rise in SMR for pulmonary disease (SMR 2.01 95% CI 1.00-3.60) 10 years after diagnosis but no rise in mortality overall\textsuperscript{30}.

2.2 Extra-intestinal malignancies.

There are several reports of extraintestinal malignancies associated with UC but there is no cogent reproducibility in these studies regarding a site-specific extra-intestinal cancer correlated to UC\textsuperscript{31-38}. In a Swedish population-based study from Uppsala, Ekbom et al found an increased risk of connective tissue and brain cancers in patients with extensive colitis at diagnosis\textsuperscript{31}. These results were not verified in another Swedish study from Stockholm by Karlén et al. In this study, 1547 patients were followed a mean 16.5 years. A significantly increased standard incidence ratio (SIR) was found for liver and hepatobiliary cancers in men (SIR 6.0; 95% CI 2.9-11.1) associated with primary sclerosing cholangitis. On the other hand, a significantly decreased risk for pulmonary cancer was observed (SIR 0.3; 95% CI 0.1-0.9)\textsuperscript{32}. Mellemkjaer et al. also reported a significant increase in the relative risk of hepatobiliary cancer from a Danish population-based study\textsuperscript{33}. A fourth population-based study performed by Bernstein et al from Canada, again showed an increased risk for patients with UC to be affected with hepatobiliary tract tumors (3.96; 95% CI, 1.05-14.9). In this study, there was no increased cancer risk overall for UC\textsuperscript{34}. Thus, three out of four population-based studies show an increased risk of hepatobiliary tract tumors for patients with UC.

Primary sclerosing cholangitis (PSC) is the main extra-intestinal manifestation with prevalence varying from 1.6 to 7.5% in UC\textsuperscript{35-38} and patients with PSC are affected with UC in 70-80%\textsuperscript{39,40}. Furthermore, patients with UC and PSC have been shown to be at increased risk of developing colorectal neoplasia\textsuperscript{40-42}. The explanation for this phenomenon could be that those patients with PSC and UC seem to have a more quiescent form of UC and therefore are missed in the surveillance programmes. An increased risk for cholangiocarcinoma in patients with PSC is well established\textsuperscript{43,44}. In a Swedish national-based study including 604 patients with PSC, a SIR for hepatobiliary
carcinomas was found to be 161 (95% CI 120-210). This would explain the observed increased risk for patients with UC to be affected with hepatobiliary tract tumors in the three population-based studies cited above. It could be of value, not only to scrutinize patients with PSC for UC, but also to examine patients with UC more closely for PSC.

2.3 Colorectal cancer risk

In the early study from Devroede et al, a CRC risk of 20% per decade, after the first decade of disease, was reported. There has, since this report, been numerous articles published concerning the risk of CRC in patients with UC. To extract accurate and cogent conclusions from these studies is not easy since they differ in many variables. For example; definition and distribution of disease extent, geographical location, population-based vs. referral centres-based, severity of inflammation, treatment policy, indications for and type of surgery, duration of disease, follow-up time and methods of analysis and giving account of the result. Since referral centres per definition come to see more complicated courses of UC, it is plausible to presume that the CRC incidence would be increased in such studies as compared with population-based studies.

Gyde et al performed a study that comprised three different geographically defined areas in Sweden and England. Eight hundred and twenty-three patients with onset of UC between 1945 and 1965 were included and followed for more than 17 years, 486 of these patients were classified as having extensive colitis. Life table analyses in extensive colitis showed cumulative risks of 7.2% (CI 3.6-10.8) at 20 years from onset of disease and 16.5% (CI 9.0-24.0) at 30 years from onset. In another study from Uppsala comprising 3117 patients of whom 1045 were diagnosed with extensive colitis, a similar cumulative risk of 8.0% 20 years after diagnosis was found. Jess et al showed in a population-based study from Minnesota, USA comprising 378 patients, an increased risk of CRC in patients with extensive colitis of SIR 2.4 (95% CI 0.6-6.0) but no increased risk was seen in patients with a limited extension of UC. The corresponding figures for all patients with UC in the study by Karlén et al was 4.1 (95% CI 2.7-5.8) and in the study from Ekbom et al 5.7 (95% CI 4.6-7.0). Eaden et al reported in a meta-analysis the risk of CRC in UC overall to be 2% at 10 years, 8% at 20 years and 18% at 30 years and indicated an increase of risk over time. The authors analysed 116 different studies, with different study designs including referral centre studies. On the other hand, a recent population-based study from Denmark showed no increased risk in CRC and another recent study from England showed a low increased risk of cumulative CRC incidence of
2.5% at 20 years disease and 7.6% at 30 years disease\textsuperscript{52}. Even if only population-based studies are considered, there is no straight answer to what the risk is. The studies from Denmark and USA show a lower risk\textsuperscript{50,51} compared to the studies from Canada, England and Sweden\textsuperscript{54,47,48}. The explanation to this is not clear but could be due to factors such as colectomy rate, compliance to medication and surveillance, or other factors.

In the studies from Uppsala and Minnesota, a decrease in CRC was found in patients with onset of disease in more recent decades\textsuperscript{48,59}. The explanation could be that these patients have had access to modern medical treatment during the entire course of their disease. This assumption is supported by a case-control study of the patients who were affected with CRC in the Uppsala study compared to age-, sex-, calendar year of diagnosis- and duration of UC-matched patients without CRC\textsuperscript{53}. Treatment with sulfasalazine for at least 3 months was associated with a significant protective effect with a relative risk of 0.38 (95% CI 0.20-0.69) independent of disease activity.

The beneficial outcome regarding sulfasalazine medication and CRC is further supported in two studies from the UK\textsuperscript{54,55} and in one study from the USA\textsuperscript{56}.

2.4 Risk factors for CRC in UC

Extension, duration and young age at onset of UC have been regarded as the classic risk factors for CRC in UC. Several studies support that long duration and extended colitis is associated with an increased risk for CRC\textsuperscript{19,46-49,57}. More diverse results have been reported on whether onset at young age is a risk factor per se or if it could be an outcome of long duration of disease\textsuperscript{19,45,47,48}. A more recently identified risk factor would be UC in combination with PSC\textsuperscript{40-42,57,58}. Lindberg et al observed that CRC in patients with concomitant PSC and UC predominately located in the proximal part of the colon could suggest that toxic bile plays a role in the carcinogenic process in patients with PSC\textsuperscript{58}.

Another possible risk factor is a family history of CRC\textsuperscript{56,59}.

Apart from the above mentioned risk factors, which are obtained from the patient’s history, there are microscopic factors to consider. The most accepted and widely used in daily clinical practice is the dysplasia of colorectal epithelium. Epithelial changes associated with CRC have been known for the last 4-5 decades\textsuperscript{50,61} but the classification used today was set by an international group of pathologists in 1983\textsuperscript{52}. This classification categorises epithelial changes as negative, indefinite or positive for dysplasia. The term "dysplasia" should be reserved for changes in the epithelium that are unequivocally neoplastic. The positive category is further divided into two sub-categories; high-grade
dysplasia (HGD) and low-grade dysplasia (LGD). In spite of this classification, there are problems with intra- and especially inter-observer inconsistency among pathologists.\(^{63}\) Another problem is that the interpretation and validation of the concept of dysplasia varies among gastroenterologists.\(^{64}\) The term DALM (dysplasia associated lesion or mass) was introduced by Blackstone et al in 1981.\(^{65}\) The term was used for a single polypoid mass, a plaque-like lesion, or multiple polyps with either LGD or HGD and is regarded as an additional risk factor for CRC. There is no dispute about the high malignancy potential of HGD and such findings should lead to a recommendation of surgery.\(^{66,67}\) The import of LGD is, on the other hand, not clear. Two recent studies with similar methodology, one from USA and the other from the UK, showed different results.\(^{68,69}\) Ullman et al found that the rate of neoplastic progression in LGD in flat mucosa was 53\% at five years.\(^{69}\) The other study performed by Lim et al found that progression from LGD to HGD or CRC occurred in 10\% of the patients at ten years. The same figure in a control group with no LGD at start of the study, was 4\%.\(^{69}\) Thus, there is no cogent consensus of the definite progression potential of LGD.

Hammarberg et al described in 1984 findings of abnormal cell content in samples from colonic mucosal samples from patients with UC using DNA-flow cytometry.\(^{70}\) Later on it has been confirmed that DNA aneuploidy is frequently associated with neoplasia (dysplasia and CRC) in UC.\(^{71-75}\) DNA aneuploidy is a useful complement to dysplasia as a marker in surveillance, and is analysed as a complement to histopathology in some centres.\(^{73,75}\) Specificity and sensitivity are not, however, at the standard that aneuploidy itself could predict neoplasia.\(^{75,76}\)

Inflammation is today believed to be important in CRC carcinogenesis in UC.\(^{17,77,78}\) Inflammation causes repetitive damage to the mucosal cells which induces chromosomal instability, oxidative stress and p53 activation, all of which contributes to the DNA damage seen in UC.\(^{79}\)

To minimise inter-observer variability it would be highly desirable to find molecular factors predicting neoplasia in UC to help identify patients at risk of CRC. Even if advancement has been achieved in the understanding of the carcinogenesis of CRC in UC, no better premalignant marker than dysplasia has yet come into clinical practice.
2.5 Surgery in UC

Even if medical treatment is fundamental in the care of patients with UC, there is a
definitive role for surgery and approximately 30-45% of the patients will at some point
require surgical treatment. Indications for surgery can be divided into elective
indications and emergent indications. Examples of elective indications are
fulminant/unresponsive nature of first attack, therapy resistant disease, side effects/non-
compliance with medication and neoplasia in the colorectal mucosa. A more infrequent
indication for colorectal surgery is the appearance of extraintestinal manifestations.
Cutaneous, peripheral articular, ocular, haematological, and vascular manifestations have
been reported to improve after surgery but surgery in patients with concomitant PSC
has no beneficial effect on the liver disease.

Emergent indications for surgery are fulminant colitis, toxic megacolon, haemorrhage
and perforation. The risk of colectomy is high if therapeutic response is absent after three
days of intensive medical treatment.

Colectomy rates have varied widely over the years and from study to study. In the County of Copenhagen, 9% of the patients with UC in 1962-87 underwent
collectomy within the first year of disease as compared to 6% during the years 2003-
2005. The cumulative colectomy rate from the same area at 10- and 18 years were 23%
and 31% respectively, reported in 1985. In the County of Stockholm, the 25-year
cumulative colectomy rate was 65% in patients with total colitis compared to 45% in all
patients reported in 1990. These high numbers probably mirror the previously adopted
aggressive tactic of early prophylactic panproctocolectomy (PPC) in patients with at least
extensive colitis to minimise the risk of CRC. PPC was advocated after 10-15 years of
disease. In the study from Olmsted County, Minnesota, with endpoint in 2001, the
cumulative risk for PPC was 20.8% after 25 years. The corresponding figures from a
study from St. Mark’s Hospital, also with endpoint in 2001, showed a remarkably low
cumulative PPC rate of 9.1% at 20 years and 20.4% at 30 years. The altered approach to
minimise potential CRC, from prophylactic PPC after a set number of years with disease
to surveillance colonoscopy, as well as modern medical treatment, could be explanations
for the observed decline in the colectomy rate. The distribution among the three major
indications in Sweden is at present: acute colitis, 40%; therapy resistant colitis, 53% and
prophylaxis/cancer, 7%.
“Does this mean that I will have to have a bag on my belly?” is not an uncommon question asked by patients when being informed about their diagnosis of UC. Although people are not particularly familiar with UC, they are aware of enteric stomas. This may originate from an earlier era when the treatment of UC basically consisted of surgery and the predominant operation until the late 1970s was PPC with a terminal ileostomy. Quality of life (QOL) is impaired with ileostomy\textsuperscript{90,91} which incited the development of a continent technique in the late 1960s\textsuperscript{92}. In 1978 Parks and Nicholls introduced the ileal pouch with pouch-anal anastomosis (IPAA), which has become the gold standard in PPC today\textsuperscript{93}. Being spared an enteric stoma is an obvious advantage of this procedure, but there are also disadvantages. The procedure is often performed in two steps (and in acutely ill patients, sometimes three steps) in that a protective defunctioning stoma frequently is performed in attempt to minimise the consequences in the event of anastomotic failure\textsuperscript{94}. There is considerable morbidity perioperatively as well as postoperatively, and even a noticeable mortality connected with IPAA\textsuperscript{95}. Furthermore, a patient with an IPAA probably needs life-long surveillance of the pouch, even if the risk of neoplasia seems to be low\textsuperscript{96,97}. The functional outcome is reported to be good\textsuperscript{98,99} as well as the QOL\textsuperscript{100}, even if there are reports of pouch failures in 10% of the patients and the cumulative probability of pouchitis is found to be as much as 38% after 10 years\textsuperscript{101-103}. An alternative to the above mentioned operative techniques is colectomy with an ileo-rectal anastomosis (IRA) which implies a potential risk for malignancy in the spared rectum, and consequently, also will demand continued life-long surveillance of the rectum in situ. The procedure is on the other hand easier to perform technically, and the risk of decreased fecundity in women associated with IPAA\textsuperscript{104-107} is diminished. The functional outcome was in a comparing study between IRA and IPAA from France not shown to differ, but the authors suggested IRA to be a better alternative for patients in whom there was any doubt about the diagnosis i.e. Crohn’s disease\textsuperscript{108}. The procedure of IRA seems to be safe regarding malignancy\textsuperscript{109} and about 50% of the patients will have their rectum in situ after 10 years\textsuperscript{110}. 
2.6 **Surveillance**

The tactic to minimise the observed increased risk of CRC in UC changed during the 1970s from time-with-disease scheduled prophylactic PPC to the concept of colonoscopic surveillance. This concept is based on the assumption that CRC in UC is preceded by epithelial dysplasia in the colorectal mucosa\textsuperscript{17,60-62,111,112} and that by harvesting biopsies at regular intervals throughout the colorectum it is possible to select patients at high risk for CRC and recommend them prophylactic PPC. Even if the association between dysplasia and cancer seems to be clear\textsuperscript{112}, there is no cogent correlation. In other words, the absence of dysplasia does not exclude CRC\textsuperscript{52,66,113,114} but with close colonoscopy intervals, an early, curable stage of CRC would be detected\textsuperscript{114-117}. On the other hand, a recent Danish study has shown that patients with UC and concomitant CRC have a stage distribution similar to patients with CRC without UC and that the prognosis of CRC is poorer for patients with UC\textsuperscript{118}. An explanation for this could, according to the authors, be that there were no information available whether the patients had participated in surveillance programmes or not.

Surveillance for CRC using colonoscopy is arduous for the patient as well as for the medical system. It is important to include all patients that could benefit from surveillance but this task has proved to be difficult in some studies\textsuperscript{119-121}. The explanations for this are not clear but could be due to the demands of the bowel preparation routine or the colonoscopy procedure itself, in addition to people migrating or getting lost in the follow-up. Another contributing factor could be that patients are poorly informed about the risk of CRC and surveillance routine\textsuperscript{122-123}.

The concept of surveillance is not unquestioned. Some authors imply that the procedure is not cost-effective and does not work mainly due to the limited sensitivity and specificity in the diagnosis of LGD and its subjectivity to gross inter-observer error\textsuperscript{124,125}. Considering that the alternative to surveillance would be the earlier strategy of prophylactic PPC after 10 years of disease or simply “wait and see”, surveillance seems to be the favourable. As mentioned above, several studies have indicated that colonoscopic surveillance programmes are beneficial for the patient in respect to mortality from CRC but there is no clear evidence that colonoscopy surveillance prolongs survival in patients with extensive UC\textsuperscript{126}. Such evidence would be hard to obtain since it would require a randomised clinical trial comprising a large number of patients with UC, comparing surveillance with no surveillance. Even if there is no clear evidence of the effectiveness of surveillance, there are guidelines published by different societies
advocating such a strategy\textsuperscript{127-129}. One of the factors that lead to the guidelines designed by Eaden and Mayberry in the UK was that an audit of gastroenterologists showed that surveillance programmes were disorganised and inconsistent\textsuperscript{127}. In fact, studies have shown that the attitude toward surveillance and the awareness and knowledge about dysplasia among gastroenterologists differs and is sometimes deficient\textsuperscript{130,131}.

2.6.1 Surveillance in practice.

There are several obstacles to overcome in reaching satisfactory results in a surveillance programme. First, the physician, as well as the patient, has to be convinced of the benefit of the procedure which entails education/information\textsuperscript{122,131} including complete information and understanding of the consequences in case of findings of premalignant signs (HGD, DALM, repeated LGD). This is important because it is not unusual to find these alterations in healthy patients with quiescent disease. When this occurs the patient has to undergo major surgery, the outcome of which will effect his daily life. Every patient regarded to be at high risk, i.e. having extensive longstanding UC, early onset disease and patients with concomitant PSC, has to be enrolled and compliant.

According to the findings that the risk of CRC becomes greater than that of the general population after 8-10 years from the onset of symptoms (not from date of diagnosis)\textsuperscript{19,45,47,48,114} colonoscopic surveillance should be initiated at this time\textsuperscript{66,112,117,127,129}. The start of surveillance should be calculated from the onset of symptoms and not from the date of diagnosis since the disease can be quiescent and many patients do not receive their diagnosis until after many years have passed. This is particularly true in patients with concomitant PSC, who should be enrolled in the surveillance program upon diagnosis\textsuperscript{127}. The recommendations for surveillance intervals differs slightly between published guidelines but a common procedure adopted in Swedish studies, including our centre, is biannual investigation of patients negative for dysplasia and having a time of disease less than 20 years. Thereafter, annual colonoscopies are performed\textsuperscript{114,132,133}. Regarding patients with left-sided colitis, some authors suggest that surveillance can be initiated after 15-20 years of disease\textsuperscript{127}.

The question of how many biopsies should be harvested from the flat mucosa to minimise the sampling error and produce an acceptable degree of sensitivity has not been studied. Awareness of the fact that up to 40 biopsies only covers 0.1% of the colorectal mucosa is cause for reflection about the sensitivity of surveillance\textsuperscript{112}. The British guidelines recommend taking two to four biopsies every 10-cm of the entire colon and additional
samples from the rectosigmoidal area\textsuperscript{127}. On the other hand, there are reports supporting mucosal harvest from 6-10 different colorectal areas as sufficient to disclose dysplasia at an adequate level to reveal potential malignancy\textsuperscript{52,66,112-115,121}. To eliminate the difficulty of differentiating reactive change from dysplasia in the biopsy specimens it is important to perform the colonoscopy during remission\textsuperscript{62}.

The strategies regarding findings of HGD in the biopsy specimens are not questioned. Studies have shown that such findings in flat mucosa followed by immediate surgery revealed CRC in 42-67\% of the colectomy specimens\textsuperscript{60,67}. From the same literature review by Bernstein et al\textsuperscript{67} comprising 1225 surveillance patients we learn that if a DALM, regardless of grade of dysplasia, led to surgery then CRC was found in the operative specimens in 43\%. On the other hand, more recent studies have shown that the genetic alterations in DALM and in non-colitis sporadic adenomas are similar\textsuperscript{134} and that the outcome from endoscopic treatment i.e. polypectomy could be sufficient\textsuperscript{135-137}. In a recent larger study performed by Vieth et al, 148 patients with UC were followed a mean 6.0 years after colorectal adenomas were diagnosed and treated in different ways. Eighty-seven patients were treated with endoscopic removal of the adenoma, 60 patients did not undergo endoscopic or surgical removal of the diagnosed adenoma and one patient underwent PPC. In the group of patients that had been treated with polypectomy, 4.6\% developed neoplasia in other colorectal segments but none in the same segment in which the adenoma was located. In the group of patients with no treatment, 48.3\% developed neoplasia in the same colon segment\textsuperscript{137}. Thus, it seems that endoscopic removal of adenomas followed by close surveillance could be an alternative to the earlier imperative recommendation of PPC. Accordingly, the true fate of every DALM is not yet revealed\textsuperscript{138}.

Regarding findings of LGD in flat mucosa, the guidelines recommend PPC in cases of repeated and/or concomitant findings in different segments during the same investigation. These recommendations rely on reports indicating a progression rate in five years from LGD to HGD or CRC of 50-55\%\textsuperscript{66,68,139} and that patients that have undergone surgery on the indication of LGD can have an unrecognised synchronous CRC in up to 20\%\textsuperscript{67,139}. In the most recent report from St. Mark’s Hospital’s 30-year experience in surveillance, 39.1\% of the patients with LGD developed HGD or CRC\textsuperscript{52}. Though, as mentioned earlier, there are other studies showing a lower rate of progression\textsuperscript{69,140} and the option of shorter surveillance intervals in case of findings of LGD is a viable option for patients that hesitate to be operated on with a PPC.
The technique of surveillance of patients with UC started with, and has developed and been refined parallel to the technical advancement of the flexible endoscope. Recent advances in the technical field are variable stiffness of the endoscope and magnetic endoscope imaging. The objective of these innovations was to avoid looping of the endoscope and to make straightening of these loops easier. Another advantage would be pinpointing lesions to a more accurate location, avoiding fluoroscopy. Studies indicate support of these hypotheses but the main value seems to be in the education of colonoscopists\textsuperscript{141-147}. My personal view is that both the variable stiffness endoscope and the magnetic endoscope imaging are of great value regarding facilitation of the investigation as well as reducing pain for the patient.

Another new technique, recently introduced, is the use of chromoendoscopy (CE). The concept of CE is that the detection of flat adenomas, intraepithelial neoplasia and early CRC could be improved using a dye sprayed on the colorectal mucosa. The group from St. Mark’s Hospital performed a study in which one hundred patients underwent one regular colonoscopy with a total of 2904 non-targeted biopsies taken and no dysplasia was found. Thereafter, a second colonoscopy was performed using the dye-spraying technique and 157 targeted biopsies were taken with a finding of nine dysplastic lesions of which seven were visible only after spraying indigo-carmine\textsuperscript{144}. These findings were supported by a study by Hurlstone et al.\textsuperscript{145}.

An additional tool used to enhance the yield of the colorectal sample harvest and perhaps decrease the number of them could be the magnification endoscope\textsuperscript{146,147}. These techniques seem to have a potential substantial value in the surveillance of patients with UC but the beneficial outcome reported may depend on the fact that the colonoscopies, in at least the studies from the UK, were performed by stated experts in colonoscopy\textsuperscript{144-146}. 
3. AIMS OF THE PRESENT STUDIES

The aims of this thesis were to investigate and analyse the outcome in an unselected population of patients with ulcerative colitis derived from a defined catchment area and thereby contribute to the collective knowledge of the disease by:

1. Analysing the incidence of UC in the catchment area and calculating if patients are at an increased risk of being afflicted with CRC.

2. Investigating mortality in UC in the catchment area.

3. Evaluating the efficiency of a colonoscopic surveillance programme with respect to outcome of mortality, CRC and dysplasia.

4. Determining the presence of DNA aneuploidy in colorectal mucosa, its association with and temporal relationship to histopathological alterations and its value as a premalignant marker.

5. Investigating the outcome for patients that underwent limited resection of the colon and/or rectum instead of PPC, with special attention to those with neoplastic changes.

6. Studying the incidence of DNA aneuploidy, dysplasia, CRC and PSC in patients with early onset of UC.

7. Analysing the expression of CK7 and CK20 in colorectal mucosa in patients with ulcerative colitis, correlating the findings to diagnosis of dysplasia, aneuploidy and inflammatory activity and evaluating the potential value of cytokeratins in relation to carcinogenesis.
4. MATERIAL

4.1 Demographic background

All known patients with UC from the catchment area of Örnsköldsvik Hospital (Figure 1) have been invited to attend an ongoing colonoscopic surveillance programme that started in 1977 at the Department of Surgery at this hospital. The patients recruited to this program were found by scrutinizing the diagnostic files from the Departments of Surgery, Internal Medicine and Pediatrics as well as repeated inquiries to the general practitioners in the area from 1961 to 1983. In addition to this historical search patients were continually enrolled in the programme. The studies presented here are based on data collected from this programme. Seven additional patients are included in paper IV. These patients were identified in the historical search and reported on in an earlier study\textsuperscript{148} but have not participated in the surveillance programme since they either moved out of the area or were radically operated before surveillance colonoscopy started.

The hospital is a community hospital and is situated in the northern part of Sweden about 110 kilometres from the nearest larger medical centre, Umeå University Hospital. The catchment area is well defined but the population has declined from about 70 000 to 60 000 inhabitants from 1961 to 2005. During this period, no PPC has been performed on the indication of scheduled prophylactic intervention after 10 years of UC. This is principally due to the low cancer risk reported in an earlier Swedish study\textsuperscript{149}. The management of the patients has mainly been accomplished in our hospital through close co-operation between the departments of Surgery, Pediatrics and Internal Medicine. A small number of pediatric patients have been referred to the Karolinska University Hospital, Huddinge, and during the last 10 years patients have been referred to the Department of Surgery at the University Hospital of Umeå for planned IPAA.
Figure 1. The catchment area of Örnsköldsvik Hospital
4.2 Patients
All patients included in this thesis have, for a shorter or longer period, resided in the catchment area of Örnsköldsviks Hospital. The diagnosis of UC has been established by a history of diarrhoea with or without bloody stool and signs of ulcerated fragile colorectal mucosa found during endoscopy, together with histopathological findings in the harvested biopsy samples. In one patient the diagnosis was established by autopsy.
Total colitis (TC) was defined as morphologically altered mucosa concordant to colitis to or beyond the hepatic flexure. Left-sided colitis (LC) was defined as an involvement to a lesser degree than this but to a greater degree than restricted rectal involvement. Patients with inflammation limited to the rectum i.e. proctitis, were excluded on the grounds that this group of patients has little or no increased risk of CRC compared with the background population. There has been no routine follow-up of these patients as recommended by guidelines with a screening colonoscopy after 10 years of disease to evaluate a possible progression of the inflammation.

4.2.1 Prevalence and incidence.
On December 31, 2005, 157 patients, 63 women and 94 men with ulcerative colitis were known in our catchment area, giving a prevalence of 261/100 000 inhabitants. The annual incidence of UC in the period 1977-2005 is calculated to be 7.6/100 000.

4.2.2 Paper I
This series comprised 211 patients of whom seven were later found to have Crohn’s disease. Of the remaining 204 patients (82 women), 135 had TC and 69 LC. At the end of the study, 90 patients (including the patients who had undergone surgery) had had TC for more than 10 years. The age at onset of disease was 3-80 years (mean 32). Twenty-three patients moved out from the area and 13 patients were excluded due to poor compliance. Thirty patients died during the study period (28 from intercurrent disease), six left the programme because of old age (>80 years) and one patient due to intercurrent disease (heart disease). During the study period with start in 1977 to endpoints, which were PPC, death from any cause, relocation of the patient from the area and termination of the study in 31 December 2002, 122 patients entered and 97 left the programme.
4.2.3 Paper II
The cohort in this report comprised 147 patients that were investigated with colonoscopy on at least one occasion. During the study period 1984-1997, ten patients moved out of the area and therefore were not included. Two patients with left-sided colitis and quiescent disease left the programme.

4.2.4 Paper III
During the study period in this report which was from 1977 through 2003, 210 patients (84 women) were comprised in the surveillance programme, 141 had TC and 69 LC. At the end of the study, 101 patients had had TC for more than 10 years. Thirty-one patients died, of whom 29 from intercurrent disease and two from CRC. Twenty-five patients moved out of the catchment area and 14 patients were excluded because of poor compliance. As verified with the Swedish Oncologic Centres, there were no reports of CRC among these patients. Six patients left the programme due to advanced age and one because of intercurrent disease. End-points in this study were the same as in paper II except that the date of closure was 31 December 2003.

4.2.5 Paper IV
Forty-six patients (19 females) with onset of UC at age 18 years or younger were reported in this study. The patients in the study were collected from our surveillance programme and from a previous study. In this earlier report, patients with early onset of UC were found by searching medical reports from this hospital as well as by repeated questions to the general practitioners in our area. Seven patients from this report did not participate in the surveillance programme due to either radical surgery or relocation from the area before the programme started. These seven patients are included in paper IV resulting in a study period from 1961 through 2005. Thirty-eight patients were classified as TC and eight LC. During the 45 year long study period, 20 patients moved out of the area and information about the course of their disease was obtained via personal letters to the patients and/or medical records from their respective hospitals. One patient did not respond to written correspondence but no medical records regarding UC were found, thus indicating no complications to UC.
4.2.6 Paper V

Fifty-one patients with UC collected from the surveillance programme and five controls were included in this study. Tissue were selected from seven groups of patients consisting of; normal control biopsies (5), inactive colitis (10), active colitis (10), findings of LGD (10), HGD (6), aneuploidy without dysplasia (4), aneuploidy with subsequent dysplasia (6) and five patients with CRC. Five patients were represented in two groups but with different biopsies.
5. METHODS

5.1 The surveillance programme.

The ongoing surveillance programme started in 1977 and was running at full extent from 1980. Patients with onset of disease before 1977 were, as earlier mentioned, identified by scrutinizing medical records from the hospital as well as by requests sent to general practitioners. Before colonoscopy was available, barium enema and rigid sigmoidoscopy with biopsy were used to establish UC. The colonoscopic surveillance in the earlier era included barium enema and colonoscopy performed at onset of the disease or upon entering the programme. Barium enema later became more unusual in the process of obtaining the diagnosis of UC. Colonoscopic surveillance started after 10 years duration of disease until 1988 and thereafter, if TC was established initially, after 6 years of disease. This change in routine was made because of a fatal case of CRC that was diagnosed 9 years after initial diagnostic colonoscopy.

After 20 years of disease duration, colonoscopy was performed annually. The surveillance programme has basically had the same formula for patients with LC, even if some patients with quiescent disease and repeatedly negative for dysplasia samples, have been investigated every fourth year in their second decade and bimonthly in their third decade of disease. The usual routine to execute the colonoscopies was to gather patients to a “colonoscopy week”, which was performed twice a year. Five colonoscopies/day were performed.

5.2 Colonoscopies, histopathologic evaluations & DNA analysis.

Principally three different investigators performed a total of 1035 colonoscopies. During colonoscopy, two samples from flat colorectal mucosa were taken for histopathologic evaluation at six locations; caecum, ascendant-, transverse-, descendant-, sigmoid colon and rectum. In addition to these sites, particular attention was paid to areas of mucosal irregularity and plaque-like lesions that might represent macroscopic areas of dysplasia. Since 1984, two additional biopsies have been taken at each site to evaluate presence of DNA aneuploidy. The biopsy specimens were fixed in 4% phosphate buffered formaldehyde, paraffin-embedded, sectioned and stained with hematoxylin-eosin. Dysplasia was diagnosed and classified principally by one pathologist, as high-grade, low-grade, and indefinite for dysplasia (ID) according to an international classification\(^2\). Since 2001, two pathologists have evaluated the samples. To analyse the DNA content,
the mucosal samples were minced into small pieces and immediately frozen to -80°C in DMSO, stored and at time for analysis, once again minced and DNA stained according to Vindelof et al\textsuperscript{150}. The nuclear suspension was evaluated with respect to ploidy using FACScan instrument (Becton Dickinson, Franklin Lakes, New Jersey, USA). DNA indices were calculated by the Cellfit software (Becton Dickinson). Samples with more than one peak in the histogram were judged as aneuploid. In paper V the biopsy specimens from one representative colonoscopic examination were reviewed. When extracting samples for the study, all samples were re-reviewed in a single serial by one pathologist. From each formalin-fixed and paraffin-embedded tissue sample, a 4μm section was cut, dried, dewaxed and rehydrated. The following immunohistochemical staining procedure was performed in a semiautomatic staining machine (Ventana ES, Ventana Inc., Tucson, AZ, USA). The slides were enzymatically pre-treated with Protease 1 (Ventana Inc., Tucson, AZ, USA). The primary antibodies used were monoclonal cytokeratin 20 and cytokeratin 7 (DAKO A/S, Denmark) diluted 1:500 and 1:1000, respectively.

All stained slides were interpreted by one observer, who was unaware of the results from other analyses at the time of evaluation. The evaluation of the expression was performed using semi-quantitative scales. The scale used for all analyses was: score 0, score 1, 0-25% positive cells; score 2, 25-50%; score 3, 50-75% and score 4, 75-100%. The expression was evaluated both at the crypt base and at the apical part of the crypt in the colonic mucosa while colorectal cancer samples were evaluated both at the luminal border and at the invasive margin as described by Palmqvist et al\textsuperscript{151}.

5.3 Operations.

Fifty-eight major colorectal operations were performed on 51 patients. Indications for surgery were severe therapy-resistant disease (TRD), repeated findings of LGD in flat mucosa or findings of HGD, DALT or CRC. If one of these conditions was established, the patient was advised surgery. The patients were informed of the different surgical procedures, i.e. IPAA, ileostomy, colonic and/or rectal resection, and their pros and cons. Some of the patients with pre-neoplastic mucosal findings rejected surgery as long as there was no established CRC. Even if CRC was diagnosed, some patients did not want to undergo PPC and therefore a colonic and/or rectal resection was performed. The choices of selective resection or PPC were thus mainly founded on the patients’ informed preference. The choice of IPAA or permanent ileostomy was mostly dependent on the
age of the patient and competence of the anal sphincter. The preference of the patient was of course contributory. Most of the operations were carried out at Örnsköldsviks Hospital but the procedures of PPC with IPAA (12 operations) have mainly been performed at Umeå University Hospital.

5.4 Diagnosis of PSC.
The Department of Surgery at Örnsköldsviks Hospital has not diagnosed any case of PSC. This diagnosis was given in the medical records obtained from the Departments of Pediatrics and Internal Medicine at Örnsköldsviks Hospital, Umeå University Hospital and Karolinska University Hospital, Huddinge. As verified by these records, the diagnosis of PSC was obtained by using magnetic resonance cholangiopancreatography (MRCP) and/or endoscopic retrograde cholangiopancreatography (ERCP) and/or liver biopsy.

5.5 Statistical analyses.
In this thesis, but not in the separate papers, we have calculated the relative risk for development of CRC in our cohort of patients. Accordingly the statistical calculations were performed on the cohort, as it was constituted December 31 2005. The person-years were calculated from onset of disease to one of the following endpoints: diagnosis of CRC or PPC, relocation from the area (patients with adult onset), leaving the protocol because of advanced age or intercurrent disease, death or endpoint on date of 31 December 2005. Calculations were done using the program PYRS:\textsuperscript{152} Age and calendar year were used as stratification variables in the calculation of person-years. Age was defined by 5-year strata and calendar time by 1-year strata. The expected number of cases was calculated by multiplying the sex-, age- and calendar-specific incidence rates in the reference population by the corresponding numbers of person-years in the cohort. The Swedish northern region was used as reference population. The standardised incidence ratio (SIR) was defined as the ratio between the observed and the expected number of cases.

Kaplan-Meier curves were calculated for sex, early onset, colitis extent, findings of aneuploidy and findings of LGD to analyse if there were any differences regarding CRC between the variables (log rank test). Thereafter a multivariate Cox regression analysis was performed using backward elimination. The Statistical Package for the Social Sciences (SPSS), version 14, was used in the statistical analyses.
5.5 Ethical considerations.

The studies in this thesis are based on data extracted from the clinical ongoing surveillance programme at Örnsköldsviks Hospital. The studies were performed in accordance with the principles stated in the Declaration of Helsinki and approved by The Regional Ethical Review Board, University of Umeå.
6. RESULTS

6.1 General results.
In the period from 1977 to December 31, 2005, covering the time for the included studies in this thesis, there were 223 patients diagnosed with UC in our catchment area. Nine of these patients later had, their diagnosis changed to Crohn’s disease. One hundred and forty-six patients had TC (92 men) and 68 (29 women) LC. At the end of the period, the prevalence for UC was, including those who had undergone PPC, 261/100 000. The incidence had been 7.6/100 000 per year. Twenty per-cent of the patients had their onset of disease before age 19 and the mean age of onset of disease was 32 years. One hundred and eight patients left the surveillance programme because of relocation from the area, advanced age/intercurrent disease, PPC or death by any cause.

6.2 Mortality.
During the period 1977-2005, four patients died of UC-related causes. In 1980, a male patient aged 37 years died of fulminant colitis after 10 years of disease. The patient had been proposed surgery because of the severity of his symptoms and findings of dysplasia (LGD two years earlier) but refused. At autopsy two colorectal cancers were found. Another patient, a female aged 38 years, died in 1987 of caecal cancer 9 years after initial diagnostic colonoscopy. The cancer was clinically diagnosed before the first surveillance colonoscopy and thereafter the routine was changed in that surveillance was started 6 years after onset of disease. A third patient, reported in Paper IV, was diagnosed with UC at age 8 and was suspected to be afflicted with PSC at age 22. He did not participate in either colonoscopy surveillance or ERCP at that point and died from cholangiocellular carcinoma 7 years later.
The fourth patient, a thirteen-year-old boy, never enrolled in the surveillance programme, died perioperatively in 1978 in a first devastating fulminant attack of UC. Thirty patients died of unrelated intercurrent diseases. Thus four, (1.9%) of 214 patients with UC died because of the disease.

6.3 Colorectal cancer (Paper I).
Nine colorectal cancers were diagnosed in eight patients (two with LC). The time from onset of disease to the diagnosis of CRC varied from 8 to 47 (mean 27 and median 30) years. Five of the carcinomas were located in the rectum, one in the sigmoid colon, one in
the transverse colon and two in the caecum. Five of them were classified as Dukes’ C and four as Dukes’ B. Three of the patients were aged 70 or older. In two other patients the cancers were diagnosed before the first scheduled surveillance colonoscopy. Three of the patients did not have any findings of preceding dysplasia before CRC was diagnosed. One patient had a DALM known for 18 years before the diagnosis of carcinoma could be established and she gave her consent to surgery\textsuperscript{138}. Two more patients had findings of DALM, 13 and 14 years respectively, before CRC was diagnosed. No additional CRC was diagnosed after the endpoint of the study reported in Paper I (Fig 2). In all our patients, an increased risk of CRC with a SIR of 4.32 (95% CI 1.86-8.52) was found. The cumulative incidence of CRC by colitis duration was 0.9% at 10 years, 1.4% at 20 years, 1.9% at 30 years and 3.3% at 40 years. In uni-variate Kaplan-Meier analyses only aneuploidy was significantly associated with CRC (p = 0.021). Sex, extent of colitis and early onset of UC showed no significance, while LGD showed borderline significance (p= 0.078). In the backward elimination multi-variate analysis including the variables above, only aneuploidy was in the end left in the model (RR 4.53, 95% CI 1.12-18.4; p = 0.034).

![Flowchart](image)

**Figure 2.** The courses for patients with CRC. Flowchart showing patients with CRC at the end of year 2002. Outcome is related to CRC as the cause of death. No further CRCs have been diagnosed since. CRC = colorectal cancer; UC = ulcerative colitis; TC = total colitis; LC = left-sided colitis
6.4 Dysplasia.

This issue is mainly addressed in Paper I from which the following results are collected. Fifty-two patients had findings of dysplasia (ID, LGD or HGD) and 5 of them developed CRC. Seventeen DALMs were diagnosed in 15 patients, 14 of whom had TC. Three of these patients developed CRC. Four of the patients did not undergo surgery; one patient with a DALM diagnosed in 1989 left the programme in 1992, one patient died one year after findings of DALM in an unrelated intercurrent disease. One patient died five years after being diagnosed with DALM of heart disease and another patient was still under surveillance six years after a single finding of DALM. Nine patients with TC were found to have HGD, two of whom progressed to CRC. In one of them, CRC was established a few months later in the same area in the operative specimen, the other patient, mentioned above, refused surgery “before cancer was a fact”, which occurred 18 years later. HGD was found 5-36 (median 19) years after onset of disease, six of the patients underwent PPC and the remaining three were operated on with different kinds of colorectal resections, specified in Paper III. No patient with HGD has died because of UC. Forty-one patients (4 LC) were diagnosed with LGD on at least one occasion. The time from onset of disease to findings of LGD varied between 1 and 46 (median 15) years. No progress regarding the grade of neoplasia was seen in 23 patients with LGD during surveillance in 1-25 (median 15) years. In 10 patients LGD preceded HGD, DALM or CRC with 1-11 (median 3) years and in eight patients LGD was found at the same time as HGD or DALM. Ten patients with findings of ID were not diagnosed with any higher grade of dysplasia.

6.5 Aneuploidy (Paper II).

The material comprised 147 patients of which 20 (19 TC) had findings of aneuploidy in their colorectal mucosa. Fourteen of these 20 patients also had morphological alterations; one CRC, five DALM, four HGD (in the patient with CRC and three in patients with DALM), seven LGD and one patient with ID. Aneuploidy was identified 5-31 (mean 18) years from onset of disease and preceded all but one of the major morphological alterations (HGD, DALM and CRC), the deviant showing aneuploidy at the same time as a DALM with HGD was diagnosed. The appearance of aneuploidy varied between seven years before to nine years after the diagnosis of LGD. In six patients, aneuploidy preceded LGD, in one patient the alterations appeared at the same time and in five patients the morphological alterations preceded aneuploidy. Regarding the 127 patients
with no signs of preoperative aneuploidy, there were four cases of CRC of which three were diploid and one showed aneuploidy in the operative specimen. Additionally there were two DALM, seven LGD and eight cases of ID. In this group of patients without findings of aneuploidy, 59% had TC compared with 95% in the aneuploidy group. Twenty-one of the 127 patients in the non-aneuploidy group had morphological alterations compared with 14 of 20 in the aneuploidy group. Of all 147 patients, there were 35 with dysplasia of whom 14 showed DNA aneuploidy (Table 1). The finding of aneuploidy was reproduced in 48 of 142 colonoscopic examinations.

Table 1. Comparison between patients with findings of aneuploidy and patients with no findings of aneuploidy regarding morphological alterations. Only the highest degree of dysplasia and cancer is considered.

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>Total-colitis</th>
<th>ID</th>
<th>LGD</th>
<th>HGD</th>
<th>DALM</th>
<th>Cancer</th>
<th>Total</th>
<th>Dysplasia or cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. aneuploidy</td>
<td>127</td>
<td>75 (59)</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>21</td>
<td>21 (17)</td>
</tr>
<tr>
<td>Aneuploidy</td>
<td>20</td>
<td>19 (95)</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>14</td>
<td>14 (70)</td>
</tr>
<tr>
<td>Total</td>
<td>147</td>
<td>94 (64)</td>
<td>9</td>
<td>14</td>
<td>0</td>
<td>7</td>
<td>5</td>
<td>35</td>
<td>35 (24)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. Only the highest degree of dysplasia and cancer is considered. ID = indefinite for dysplasia; LGD = low-grade dysplasia; HGD = high-grade dysplasia; DALM = dysplasia-associated lesion or mass.

6.6 Surgery (Paper III).

Fifty-one patients were operated on in whom 29 PPC were performed initially, including two patients with a planned two-step procedure. Additionally, ten patients had, at endpoint of the study, either been or were planned to undergo radical operation. Thus, 22 patients had their first operation performed as a resection of either a part of or the whole colon or rectum. One of these patients had a colectomy performed at the age of 15 years and a plan for proctectomy when fully grown. The other 21 patients were initially operated on with a colonic or rectal resection with no further intention of radical surgery. At endpoint, 15 patients had parts of colon or rectum in situ and three of them were awaiting proctectomy. The indications for surgery are shown in Table 2. In two of the
patients who underwent PPC at the first operation, the indications for surgery were sigmoid and rectal cancer, respectively, both Dukes’ B. Three of the patients were operated on because of DALM, two because of HGD and one because of repeated findings of LGD. In twenty patients, the indication for PPC was TRD. There were unexpected findings of one HGD and one LGD in the operative specimens from two of these patients. One patient was operated on in 1981 because of PSC. Seventeen of the patients had a permanent ileostomy and 12 an IPAA performed. Among the 21 patients that underwent subtotal proctocolectomy with no further intended surgery, there were four patients with CRC (two right-sided and two rectal cancers- one Dukes’ B and three Dukes’ C). One of the patients died six months after surgery. This patient was clinically diagnosed with a caecal cancer before her first surveillance colonoscopy. The other three patients were alive at the endpoint but one of them was scheduled for proctectomy because of findings of LGD in the remaining rectum. Three patients underwent colorectal resections because of DALM. One of them later had findings of LGD in the remaining rectum and was radically operated on and another patient was at endpoint scheduled for such an operation due to severe proctitis. All three were alive at endpoint. In 12 of 21 patients, the indication for surgery was TRD. Six of them were later operated radically, five because of severe symptoms and one because of LGD in the remaining rectal mucosa. The operative specimen from this patient revealed an unexpected carcinoma (Dukes’ B) and the patient died two years later of intercurrent disease. Two additional patients from this group also died of intercurrent disease, the other 18 patients were alive at end of the study. In two patients that were operated on with colorectal resection the indications were spontaneous perforation and colonic lymphoma respectively. Both were alive at endpoint of the study. Accordingly, none of the 21 patients under surveillance that were operated on with a colonic or rectal resection died of CRC or a metachronous cancer located in the remaining colon or rectum (Table 3). The time gained with a presumably better bowel function, from doing a limited colorectal resection instead of PPC, for these 21 patients, was 0.5-42 (mean 9.4 and median 7) years. Comparing the eight patients that were operated on with a limited resection on the indication of neoplasia with the eight patients that had a PPC performed on the same indication, one finds that the resection group gained more than six years with parts of colon in situ.
Table 2. Indications for surgery at first and second procedure. Colonic lymphoma, spontaneous perforation of the colon and sclerosing cholangitis are reported as “Other”.

<table>
<thead>
<tr>
<th></th>
<th>Total no. of patients</th>
<th>Symptom</th>
<th>LGD</th>
<th>HGD</th>
<th>DALM</th>
<th>CRC</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPC direct</td>
<td>29</td>
<td>20</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Resection</td>
<td>22</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Second op.</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

PPC = Panproctocolectomy; LGD = low-grade dysplasia; HGD = high-grade dysplasia; DALM = dysplasia associated lesion or mass; CRC = colorectal cancer.
<table>
<thead>
<tr>
<th>Gender</th>
<th>Age at first surgery (years)</th>
<th>Indication for first surgery</th>
<th>Type of colorectal resection</th>
<th>Indication for second, radical surgery</th>
<th>Part of colon or rectum in situ at end-point or death</th>
<th>Years with part of colon and/or rectum in situ</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>33</td>
<td>TRD</td>
<td>IRA</td>
<td>TRD</td>
<td>No</td>
<td>19</td>
<td>IC</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>TRD</td>
<td>CE + Ileostomy</td>
<td>Yes</td>
<td>Yes</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>31</td>
<td>TRD</td>
<td>IRA</td>
<td>Yes</td>
<td>Yes</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
<td>TRD</td>
<td>IRA</td>
<td>Yes</td>
<td>Yes</td>
<td>29</td>
<td>IC</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>TRD</td>
<td>IRA</td>
<td>Yes</td>
<td>Yes</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>TRD</td>
<td>IRA</td>
<td>TRD</td>
<td>No</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>TRD</td>
<td>CE + Ileostomy</td>
<td>Yes</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>27</td>
<td>TRD</td>
<td>IRA</td>
<td>TRD</td>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>49</td>
<td>TRD</td>
<td>CE + Ileostomy</td>
<td>Unknown</td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>33</td>
<td>TRD</td>
<td>CE + Ileostomy</td>
<td>TRD</td>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>71</td>
<td>TRD</td>
<td>CE + Ileostomy</td>
<td>LGD</td>
<td>No</td>
<td>3</td>
<td>IC</td>
</tr>
<tr>
<td>Male</td>
<td>70</td>
<td>Sigmoid stenosis</td>
<td>Sigmoid resection</td>
<td>TRD</td>
<td>No</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46</td>
<td>DLM</td>
<td>RHC</td>
<td>Yes</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>60</td>
<td>DLM</td>
<td>IRA</td>
<td>TRD</td>
<td>Waiting</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>36</td>
<td>DLM</td>
<td>IRA</td>
<td>LGD</td>
<td>No</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>37</td>
<td>Caecal cancer</td>
<td>CE + Ileostomy</td>
<td>Yes</td>
<td>0.5</td>
<td>Caecal cancer</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>Caecal cancer</td>
<td>IRA</td>
<td>LGD</td>
<td>Waiting</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>64</td>
<td>Rectal cancer</td>
<td>ARR</td>
<td>Yes</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>70</td>
<td>Rectal cancer</td>
<td>LHC + ARR</td>
<td>Yes</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>66</td>
<td>Colonic lymphoma</td>
<td>Ileo-sigmoid anastomosis</td>
<td>Yes</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>Colonic perforation</td>
<td>IRA</td>
<td>Yes</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TRD - Therapy resistant disease, IRA - Ileorectal anastomosis, CE - Colectomy, RHC - Right-sided hemicolecction, LHC - Left-sided hemicolectomy, ARR - Anterior Rectal Resection. IC - Intercurrent disease. Waiting - The patient were at the end point of the study waiting for radical surgery.
6.7 Outcome in early onset of UC (Paper IV).

The incidence for onset of UC at age 18 years or younger was found to be 6.2 children-adolescents/100,000 children-adolescents per year and 1.6 children-adolescents/100,000 inhabitants per year. The youngest patient was 3 years old, mean (and median) age at onset was 14 years (Figure 3).

![Figure 3. Age at onset of disease of 46 children and adolescents with ulcerative colitis](image)

Twenty-one major colorectal surgical procedures were performed on 17 patients (37%), in 11 of them as a PPC. Time from onset of disease to colorectal surgery was 0-32 (mean 7.8 and median 4) years. Seven patients were operated on before age 19 because of severe symptoms (Table 4). Five patients died, of whom three of intercurrent disease. One patient was diagnosed with UC at age 8 years and was suspected of having PSC at age 22 years. The patient did not participate in either colonoscopy surveillance or ERCP at that time and died from cholangiocellular carcinoma seven years later. A 13-year-old boy died within 48 hours of a devastating fulminant onset of UC. One patient (out of 46) was found to be afflicted with caecal carcinoma, thus giving a CRC- incidence of 2.2%. The patient was alive at the end of the study. Four patients (9.8%) were diagnosed with PSC, all of them had their onset of UC at age 14 or younger. As mentioned above, one of these patients died because of cholangiocellular carcinoma after 25 years duration of UC and nine years duration of PSC. Another patient was at endpoint of the study suspected to be afflicted with the same malignancy. Ten patients (22%) had findings of LGD and in
nine of them additionally DNA aneuploidy was detected. One patient had findings of aneuploidy but no LGD. One of the LGD progressed to HGD over a period of six years. In five of the patients, DNA aneuploidy preceded dysplasia.

Table 4. Indications and type of surgery. 17 patients operated on six indications.

<table>
<thead>
<tr>
<th>Indication for surgery</th>
<th>No. of patients</th>
<th>PPC – single stage</th>
<th>PPC – two stage</th>
<th>Colonic or rectal resection/transverse colostomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRD</td>
<td>12</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Dysplasia/DALM</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRC</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Perforated colon</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Megacolon</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Prophylactic proctectomy</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Legend: One patient was first operated on the indication TRD (therapy resistant disease) and later on prophylactic proctectomy and is therefore counted twice in columns 2 and 4. PPC = panproctocolectomy; DALM = dysplasia associated lesion or mass; CRC = colorectal cancer.

6.8 CK7 and CK20 as premalignant markers.

Colonic mucosa from normal controls and inactive colitis was found to be completely negative for CK7. In 9 out of 10 patients with active colitis, CK 7 was sparsely expressed in a patchy manner and connected to active epithelial inflammatory areas. Seven out of 10 patients with low grade dysplasia and 3/6 with high grade dysplasia were positive for CK7. Samples with aneuploidy without dysplasia were completely negative while 2 out of 6 showing subsequent dysplasia were positive. In the 5 cancers 2 were positive for CK7. CK20 were expressed in nearly all samples but relatively more in the lower part of the crypts in neoplasia associated lesions.
7. DISCUSSION

7.1 Patients.

The number of patients presented in this thesis is small. Is there any point in presenting the findings of investigations performed on a population of 60,000? One could argue that all knowledge contributes to the understanding of the disease. A far more important argument is that the studies are performed in a defined catchment area with a fairly stable population. All patients that have been diagnosed with UC have been followed and their compliance to regular colonoscopies has been excellent. We believe that an explanation for the good compliance is that the investigations have been performed by a limited number of colonoscopists and assistants, making the patients more confident with the unpleasant situation of colonoscopy. Another beneficial effect on compliance gained by doing studies from a small catchment area with few physicians involved is that the risk of losing patients due to administrative causes is minimised. To include or to not include? Since the surveillance programme started, it has been well known among other hospital clinics and the general practitioners in the catchment area. All of the patients included in these series have come to our knowledge by their presentation of symptoms of UC. They have all undergone colonoscopy with biopsy harvesting and accordingly been found to fulfil the criteria for UC. Patients with signs and symptoms of proctitis only have not been routinely re-examined after ten years, as suggested by guidelines. The reason for this exclusion would be the low risk for CRC in this subset of patients showed by Ekborn et al. The bearing of this finding is supported by the fact that no patient in the catchment area has been diagnosed with CRC and a concomitant unknown UC. At endpoint of the studies, the incidence rate was found to be 7.6/100,000. In a previous report from our catchment area, the incidence rate was calculated to be 4.2/100,000. The increase is in accordance with other studies but the cause is yet to be explained.

7.2 Mortality and the decrease thereof.

Four patients died from causes related to UC in a period of 29 years. Even if the cohort is small, there can and should be some conclusions drawn. The easiest way to evaluate the 1.9% cumulative mortality is to compare it with the earlier reported 4.7% cumulative mortality from the same area, covering the period 1961-1983. This comparison reflects the global reports of declined mortality rates due to UC-related causes as compared with
the discouraging early reports from Edwards, Gyde and Devroede which do not seem to be representative for UC today\textsuperscript{17-19,27,30}. Three of the four deaths in our surveillance programme occurred within the first ten years of the surveillance protocol period, which could indicate that future studies will reveal an even lower mortality rate.

7.3 Colorectal cancer and the risk thereof

The frequently cited figures from a meta-analysis by Eaden et al of 2% CRC after 10 years, 8% after 20 years and 18% after 30 years of disease could not be supported in our studies\textsuperscript{45}. During a surveillance period of 29 years (longest observation period of 47 years), there were nine CRCs found in eight patients from a cohort of 214 patients (3.7%). As mentioned earlier, there are considerable difficulties in comparing studies because of the difference in the definitions of several variables. We found an increased risk of CRC in all our patients with a SIR of 4.32 (95% CI 1.86-8.52). This risk is lower than the risk found in the study from Uppsala, and about the same as the figures found in Stockholm by Karlén et al but higher than the risk found in Minnesota and Copenhagen\textsuperscript{32,48,30,51}. Even if the depressing figures shown by Devroede et al in 1971 are to be regarded as medical history, few authors deny that there is an increased risk of developing CRC in UC\textsuperscript{19}. The observed decline in CRC over the years could be a result of different interventions such as rate of surgery, medication and colonoscopic surveillance. A high rate of surgery would of course decrease the risk of CRC. In the study from Copenhagen by Winther et al, close to 40% of the patients with pancolitis underwent surgery four years after being diagnosed with UC. The corresponding figures from Leijonmarck et al regarding the same study population as Karlén et al, was 42% after 10 years of disease\textsuperscript{86}. In our cohort, 15% of the patients with total colitis had undergone surgery within 10 years of disease. These figures indicate that rate of surgery alone does not explain the diverging risks of CRC.

Since we have not monitored the use of maintenance medical treatment in our patients, no conclusions can be drawn from our studies about the suggested beneficial impact of these drugs on CRC.

The effectiveness of surveillance colonoscopy has been questioned\textsuperscript{19,120} but nevertheless it is recommended as a strategy to diminish the risk of CRC\textsuperscript{127,129}. Surveillance colonoscopies were performed in the study from Minnesota but not in the study from Copenhagen. In the study from St. Marks’s Hospital, a referral surveillance centre, the authors present a cumulative CRC incidence of 2.5% at 20 years disease duration and
7.6% at 30 years. These figures are higher than the ones from the non-surveillance study from Copenhagen (1.1% and 2.1% at 30 years) but in the latter study, patients with left-sided colitis and even proctitis were included, which was not the case regarding the study from St. Mark’s. The comparison can thus seem to be of limited value but as with surgery rate, surveillance alone is probably not an explanation for the over-time observed decreased risk of CRC.

The location of CRC in our study was predominantly left-sided, which is in accordance with other studies, and indicates that inflammation is a factor in the development of carcinoma. Classical risk factors for CRC, which all involve inflammation, are early onset, long duration and extended distribution of the disease. We could not verify that early onset of UC is encumbered with an increased risk of CRC. In a study period of 45 years, only one of 46 patients aged 18 years or younger at diagnosis, was afflicted with CRC. This is in accordance with the findings of Langholz et al but not with the ones found by Devroede et al and Ekbom et al. An explanation for this could be a fairly high rate of surgery, in this subgroup of our cohort 30% after 10 years and in the Danish study 26%. The surgery rates in the studies by Devroede and Ekbom are not attainable.

In accordance with the accepted and earlier found predominance of CRC in patients with extended colitis, six of the eight cancer patients in our study had total colitis.

Duration of disease as a risk factor is also supported in our cohort. Median duration from onset of disease to diagnosis of CRC was 30 years (range 8-47 years) as compared with other recent studies; 12.5 (0-29 years), 12 (4-29 years) and 23.5 (11-48 years). PSC is described as a risk factor for colorectal neoplasia. We have only analysed patients with PSC in a subgroup of our cohort (patients with young age at onset of disease) and therefore could not evaluate this.

We have made statistical calculations in an attempt to identify risk factors for CRC. In these analyses, a significantly increased risk for CRC was found if DNA aneuploidy was present but the statistical analyses are considerably weak since the cohort is small with few events.
7.4 Premalignant markers and the assessment thereof.
Since there is no perfect indicator of impending CRC, dysplasia has served as the litmus test on which the decision for surgery has been made. Even if the yield of dysplasia as a premalignant marker is questioned, few clinicians would abstain the recommendation of surgery in case of findings of HGD. This leads to difficulties in evaluating the true value of HGD as a premalignant sign. All of the patients with HGD in our cohort were operated on, but one patient was followed for 18 years before a carcinoma was established. Nine patients (4.4%) developed HGD as compared with 3.2% in a study from Rutter et al\textsuperscript{52}. There were no unexpected CRCs in the operative specimens from the patients with HGD in our study, but in the study from St Marks’ there were findings of CRC in 45.5% of the operative specimens from those who underwent immediate colectomy\textsuperscript{52}. This finding gives strong support to continued recommendation of surgery for patients with established HGD.
We found LGD in 41 patients (20% of study population), in 10 (24.4%) of these patients there was an aggravation to HGD or CRC and an additional eight patients developed DALM (LGD) in a period of 1-25 years (median 15 years). The figures regarding HGD and CRC are higher than those found by Befrits et al (2 of 60 patients) and Lim et al (10% in 10 years) but lower than those found by Ullman et al (53% in 5 years) and Rutter et al (39.1%)\textsuperscript{52,68,69,140}. The divergent findings regarding the aggravation of LGD are dissatisfying because this is the instrument on which we are obliged to play. The explanation for these results could be sought in the difficulties of the histopathologic interpretations of dysplasia as well as the biopsy techniques and maybe in medical treatment. All these factors could be varying in different centres ending up with results as cited above. As a logical consequence of this, a fruitful tactic in the efforts of minimising lethal CRC in patients with UC would be to adapt ones actions to the empirical results from the local centre instead of uncritical adherence to guidelines.
Obviously a reproducible, sensitive and specific premalignant marker would be the answer to the complex of problems mentioned above. The most promising candidate has been DNA aneuploidy since findings of this frequently are associated with neoplasia. We found that DNA aneuploidy is associated with TC, long duration of disease and morphological alterations, all established risk factors for cancer development. In our study, all findings of HGD or CRC were preceded by, or present at the same time as aneuploidy. On the other hand, there were four patients with no sign of DNA aneuploidy that developed CRC.
In the statistical analyses comprising the whole study period there was a significantly increased risk of CRC in patients with DNA aneuploidy. This strengthens the view of aneuploidy as an important marker of impending malignancy. However, the events are still few and conclusions must be drawn with caution.

We believe that DNA aneuploidy has a value in surveillance of patients with UC as a complement to histological examination but cannot, should not and does not serve alone as a premalignant marker. Future studies with longer follow-up time should reveal the true nature of its value.

Cytokeratins are cell matrix proteins. In humans CK7 is related to the hepatic and pancreatic stem cells and their corresponding epithelium and appear also in complex epithelium, while CK20 is almost entirely confined to the gastro-intestinal epithelium, to the urothelium and to Merkel cells. Analyses of CK7 and CK20 have become accepted as markers discriminating liver metastasis of colorectal cancer from primary bile-duct-derived liver cancer. Studies have indicated that the expression of these cytokeratins could be related to dedifferentiated epithelial cells in adenomas and adenocarcinomas of the gastric cavity and colon\textsuperscript{154,155}. We found indications of an existing relationship between expression of both CK7 and CK20 to the presence of dysplasia of the colorectal mucosa in patients with UC. Whether the mechanism for altered expression of the cytokeratins in neoplastic development is similar to that expressed in active inflammation remains to be elucidated. Speculatively, the expression of CK7 in colorectal mucosa in patients with UC could represent a possible connection to neoplastic development.

7.5 Surgery and the outcome thereof

The increased risk for CRC in patients with UC has led to the recommendation of PPC in cases of repeated findings of LGD, HGD in flat mucosa, DALM or CRC. There are no, and there will never be, any prospective studies regarding the outcome of these neoplastic changes. The potential malignancy of dysplastic changes is well known but the true outcome of all these forms of neoplasia can obviously vary\textsuperscript{68,138,140}. Since there are drawbacks to a permanent ileostomy as well as IPAA, it could be of interest to know the outcome for patients operated on with a limited colonic or rectal resection\textsuperscript{90,91,95,101-103}. In our cohort, 21 (41%) patients were operated on with different kinds of colorectal resections. The indication for surgery in eight of these patients was neoplasia, presented as DALM (3 patients), lymphoma (1 patients) and CRC (4 patients). At endpoint, nine of the 21 patients had been or were waiting for radical surgery which is coherent to the
result presented in a study from Stockholm in 1990 in which half of the patients with an IRA still had there rectum in situ after 10 years\textsuperscript{110}. None of the patients under surveillance in our cohort died because of CRC or metachronous cancer in their remaining colon or rectum. We believe that if the medical service is flexible and prepared to adapt, i.e. shorten the surveillance intervals in respect to the histologic findings, a restrictive approach toward the decision to operate as well as the magnitude of the surgery, can be justified. This approach should not be adopted if the compliance of the patients to surveillance colonoscopy is impaired.

7.6 Colonoscopic cancer surveillance and the efficiency thereof.

Colonoscopic cancer surveillance is, as mentioned earlier, accepted but questioned. Even in surveillance programs, advanced CRCs are reported and the cost-effectiveness is argued to be low\textsuperscript{119,120,124}. If a surveillance protocol should have any prospect of effectiveness a crucial factor is compliance from the patients to the colonoscopic investigations. In the studies from Lynch and Biasco, the default rates were reported to be 26\% and 46\%, respectively\textsuperscript{119,120}. Axon studied reports from 12 surveillance protocols and found that 476 colonoscopies were needed to find one early CRC\textsuperscript{124}. This outcome was found to be poor as compared to surveillance in a normal population between the ages of 50 and 80 on a 5 yearly basis. This comparison is invalid since the endpoint in surveillance of patients with UC is not primarily the detection of an early CRC but rather findings of dysplasia and, in some centres, additional DNA aneuploidy, to guide the doctor and the patient in the decision of whether or not to operate.

In our surveillance study, we found that six patients were operated on the indication of CRC and nine patients on the indication of dysplasia. One of these 15 patients died because of advanced CRC. The patient was diagnosed with a caecal cancer nine years after the initial colonoscopy was performed and before the, at that time, first planned surveillance colonoscopy. If the alternative to surveillance is “wait and see”, speculatively 14/204 patients benefited from the surveillance protocol in our area in terms of avoiding death from CRC for the price of, roughly, 600-700 unnecessary colonoscopies in 190 patients. If the alternative, on the other hand, had been scheduled prophylactic surgery after ten years duration of disease, 90 patients would have been operated on with PPC with, at best scenario, no gain regarding survival.

Colonoscopic surveillance of patients with UC does not eliminate the risk of advanced CRC as shown in the latest report from St Mark’s Hospital in which 13 patients
developed advanced cancers during surveillance\textsuperscript{52}. There is a need for more efficient surveillance and there is a place for tools to improve our colonoscopic technique such as chromoendoscopy, magnification endoscopes and magnetic endoscope imaging. Finally, the best way to improve the efficacy would be more sensitive and specific markers of premalignancy.
8. SUMMARY

This thesis is founded on five studies performed in a period of nine years (1998-2006). The studies are covering a period from 1977 through 2005, except for paper V, in which the study period reached back to 1961. All known 214 patients afflicted with UC from the defined catchment area of Örnsköldsviks Health Service have been included. The incidence rate for UC was 7.6/100 000 per year and at the end of 2005 the prevalence was 261/100 000.

Four patients (1.9%) died from causes related to UC, which is a decrease compared to the figures found from the same area in a previous report from 1983. Eight patients were found to be afflicted with CRC, of which two were not within the surveillance protocol and three were aged 70 years or older. We found an increased risk of CRC with a SIR of 4.32 (95% CI 1.86-8.52).

In the on-going colonoscopic cancer surveillance protocol that started in 1977, compliance has been excellent. Thirteen patients had, at evaluation in the end of 2002, abstained from participation, 23 patients had moved out of the area and 28 patients had died from causes not related to UC.

Dysplasia was found in 52 patients (9 HGD, 41 LGD), of whom five developed CRC. Twenty-three of the patients with LGD were under surveillance for a median 15 years without any aggravation in the grade of dysplasia. Nineteen of the patients with dysplasia were operated on, 12 of them with PPC.

Presence of DNA aneuploidy in colorectal mucosa was determined in the cohort of 147 patients in the period 1984 - 1997. DNA aneuploidy was detected in 20 patients of whom 19 had TC, the time from onset of disease to aneuploidy was a median 18 years (5-31 years). Fourteen of these patients developed morphological alterations; in four patients aneuploidy preceded dysplasia, in three patients dysplasia were present at the same investigation and in seven patients dysplasia were found before DNA aneuploidy. In four out of five cases DNA aneuploidy was diagnosed before the appearance of HGD, DALM or CRC.

At the end of 2003, fifty-one patients had been operated on. The indications for surgery had mainly been therapy-resistant disease, HGD, CRC or repeated findings of LGD. In 29 patients a PPC had been performed as the initial operation and in one young patient, a plan for a later additional proctectomy was set up. Accordingly, 21 patients had their first operation performed as colonic or rectal resection with no further intended surgery. The
indications for initial surgery in this group of 21 patients were in four cases CRC and in three DALM. One of these patients died six months after surgery because of disseminated CRC, whereas the other five patients were alive at the endpoint of the study. In the group of patients who were operated on with a colonic or rectal resection, further nine patients were later radically operated on. Twenty-one patients gained a mean of 9.4 years with presumably better bowel function from undergoing a limited resection instead of a PPC. None of the patients under surveillance who had undergone a colonic or rectal resection died because of CRC or metachronous cancer in their remaining colon or rectum.

In the period 1961-2005 there were 46 patients (38 TC) that were diagnosed with UC at age 18 or younger. The incidence rate was 6.2 children-adolescents/100 000 children-adolescents/year and onset of disease was mean and median 14 years. Five patients died, of whom three from intercurrent disease. One patient developed CRC but no death caused by this disease was observed. PSC was diagnosed in four patients (9.8%), of whom all had their onset of UC before age 15. One of these patients died because of cholangiocellular carcinoma. In ten patients DNA aneuploidy was detected, nine of these patients also had findings of LGD, additionally one patient had findings of LGD. In one patient LGD progressed to HGD. Seventeen patients (37%) underwent colorectal surgery, seven before the age of 19.

The expressions of CK7 and CK20 in colorectal mucosa from patients with UC were studied by analysing tissue specimens from seven groups of patients and one group of normals. The groups were constituted as follows: I normals, II inactive colitis, III active colitis, IV LGD, V HGD, VI aneuploidy without dysplasia, VII aneuploidy with dysplasia and VIII CRC. No expression of CK7 was detected in group I and II. Seven out of 10 were positive for CK7 in group IV (LGD) and 3/6 in group V (HGD). No expression of CK7 was detected in group V. Samples from two of five patients with CRC expressed CK7. CK20 was expressed in nearly all samples but relatively more in the lower part of the crypts in neoplasia-associated lesions.
9. CONCLUSIONS

- The incidence of ulcerative colitis has increased in the catchment area and patients are at an increased risk of being afflicted with colorectal cancer as compared with the background population.

- Mortality in UC has decreased in the catchment area as compared with an earlier investigation.

- Colonoscopic cancer surveillance is an efficient method in diminishing the risk of lethal CRC, allowing a majority of patients to be spared colorectal surgery and it is executable in a community hospital. A prerequisite of success in surveillance is good patient compliance.

- Flow cytometric DNA analysis has a value as a complement to histological examinations in the cancer surveillance of patients with UC. The presence of DNA aneuploidy indicates a high risk for developing severe premalignant changes but cannot alone serve as a premalignant marker and should not be the sole indication for PPC.

- A limited colonic or rectal resection in patients with UC who require surgical intervention increases the time with presumably better bowel function and may therefore be an alternative to PPC without increased risk of dying from CRC.

- Patients with early onset of UC in our catchment area have not shown to be at high risk of CRC, which could be due to a high frequency of surgery. A high incidence of PSC was found, which could be a function of time, but may also serve as an incitement to amplify efforts in diagnosing this disease.

- There is a relationship between the expression of CK7 and CK20 and neoplastic development of colorectal mucosa in patients with UC. The clinical significance of these findings is not clear and further studies are needed to elucidate this question.
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