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INFLAMMATORY BOWEL DISEASE, COLORECTAL NEOPLASIA AND TREATMENT WITH URSODEOXYCHOLIC ACID IN PRIMARY SCLEROSING CHOLANGITIS

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ABSTRACT

Background: Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease closely associated with inflammatory bowel disease. PSC is progressive and ultimately leads to death or need for liver transplantation. Patients are also at high risk of developing colorectal neoplasia (CRN).

Aims: The main aim of this thesis was to study IBD in patients with PSC. We aimed to describe the phenotype of Crohn's disease (CD) in patients with PSC and to determine the risks of CRN and IBD activity before and after liver transplantation. The secondary aim was to study the drug ursodeoxycholic acid (UDCA) in patients with PSC and UDCAs effect on the development of CRN and survival in PSC.

Results: In Paper I we investigated CD in 28 patients with PSC and compared them with a matched control group of 46 patients with CD without PSC. We found that smoking, perianal fistulas, bowel strictures and small bowel involvement were rare in PSC patients. We also found a significantly increased risk for development of CRN in PSC patients (P=0.001, log rank).

Papers II and III are multicentre studies of IBD in PSC patients undergoing liver transplantation (OLT), and include all liver-transplanted Nordic PSC patients (n=439). The IBD activity was increased after transplantation and the choice of immunosuppression influenced the activity. A univariate analysis identified age <20 years at diagnosis of IBD, use of tacrolimus, and dual therapy with tacrolimus and mycophenolate mofetil as significant risk factors for worsening of IBD, whereas dual treatment with cyclosporine A and azathioprine showed a significant protective effect. The cumulative risk of any type of neoplasia in the group of patients still at risk after OLT (n=244, 36 cases of neoplasia) was higher than the corresponding number before OLT (353, 52 cases) (HR: 1.9: 95% CI 1.3-2.9, P = 0.002).

In Papers IV and V the effect of UDCA at a dose of 17-23 mg/kg in patients with PSC was evaluated using an extended follow-up of a previous randomised controlled trial. In paper IV all patients with concomitant IBD at risk for CRN were included (n=98). There was no detectable difference in dysplasia- and cancer-free survival when the groups were compared using the Kaplan-Meier method (p = 0.73 log-rank test). Paper V evaluated the effect of UDCA on long-term survival without liver transplantation. No difference in endpoint-free survival was detected between UDCA treated and untreated patients. However we found that a reduction in alkaline phosphatase (ALP) by 40% or more was associated to significantly better long-term survival in patients with PSC (P = 0.0001, log rank).

Conclusions: Our studies show that patients with IBD and PSC have a high risk of developing CRN regardless of IBD phenotype, and that the risk of CRN and IBD activity increases after OLT and appears correlated to the type of immunosuppression given. In patients undergoing OLT a shift from the present standard maintenance treatment with tacrolimus and mycophenolate mofetil to cyclosporine A and azathioprine should be considered. The evidence that UDCA improves survival in PSC or that it should be used as a chemopreventive agent in PSC-IBD is weak. ALP is a marker for disease progression in PSC and should be used in future clinical trials.

Keywords: Inflammatory bowel disease, colorectal neoplasia, ursodeoxycholic acid, immunosuppression, liver transplantation

LIST OF PUBLICATIONS

This thesis is based on the following papers that are referred to in the text by their Roman numbers.

I. **Increased risk of colorectal neoplasia in patients with Crohn's colitis and primary sclerosing cholangitis**
Lindström L, Lapidus A, Öst Å, Bergquist A.
Dis Colon Rectum, 2011. 54(11):p.1392-7.

II. **Immunosuppression after liver transplantation for primary sclerosing cholangitis influences activity of concomitant inflammatory bowel disease**
Jørgenssen K, Lindström L, Cvancarova M, Castedal M, Karlsen TH, Friman S, Schrumpf E, Foss A, Isoniemi H, Nordin A, Holte K, Rasmussen A, Bergquist A, Vatn M, Boberg KM.
Submitted 2012.

III. **Colorectal neoplasia in patients with primary sclerosing cholangitis undergoing liver transplantation: a Nordic multicenter study**
Jørgenssen K, Lindström L, Cvancarova M, Castedal M, Friman S, Schrumpf E, Foss A, Isoniemi H, Nordin A, Holte K, Rasmussen A, Bergquist A, Vatn M, Boberg KM.
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IV. **High dose ursodeoxycholic acid in primary sclerosing cholangitis does not prevent colorectal neoplasia**
Lindström L, Boberg KM, Wikman O, Friis-Liby I, Hultcrantz R, Prytz H, Sandberg-Gertsén H, Sangfelt P, Rydning A, Folvik G, Gangsoy-Kristiansen M, Danielsson Å, Bergquist A.
Aliment Pharmacol Ther 2012; 35:451-457.

V. **A reduction in alkaline phosphatase is associated with a better prognosis in primary sclerosing cholangitis**
Lindström L, Hultcrantz R, Boberg KM, Friis-Liby I, Bergquist A.
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LIST OF ABBREVIATIONS

AASLD	American Association for the Study of Liver Diseases
ALP	alkaline phosphatase
AMA	anti mitochondrial antibody
ANA	anti nuclear antibody
ANCA	anti neutrophil cytoplasmic antibody
ATG	antithymocyte globulin
CCA	cholangiocarcinoma
CD	Crohn's disease
CMV	cytomegalovirus
CRC	colorectal cancer
CRN	colorectal neoplasia
DALM	dysplasia associated lesion or mass
ECCO	European Crohn and Colitis Organization
ERC	endoscopic retrograde cholangiography
GWAS	genome wide association study
HGD	high grade dysplasia
HLA	human leucocyte antigen
IBD	inflammatory bowel disease
IND	indefinite dysplasia
IPD	indefinite probably dysplastic
IRA	ileo rectal anastomosis
LGD	low grade dysplasia
MELD	model for end stage liver disease
MMF	mycophenolate mofetil
MRC	magnetic resonance cholangiography
NLTG	Nordic Liver Transplant Group
OKT3	muromonab-CD3
OLT	orthotopic liver transplantation
PBC	primary biliary cirrhosis
PSC	primary sclerosing cholangitis
RCT	randomized controlled trial
SMA	smooth muscle antibody
UC	ulcerative colitis
UDCA	ursodeoxycholic acid

1 BACKGROUND

1.1 GENERAL BACKGROUND OF PSC

In 1927 a surgeon named Robert Miller published the first report in the English literature that described what he called “benign biliary strictures”. The patient was a 40-year-old physician who used neither alcohol nor tobacco, who fell ill with abdominal pain, jaundice and malaise. Surgery showed strictures of the bile ducts, but no biliary stones. The patient later developed blood in his stools, and recurrent attacks of colic pain and jaundice [1]. Primary sclerosing cholangitis (PSC) is a young disease; during the 50 years after Miller first described it, PSC remained a rarity and only 100 cases were published in the literature.

Today PSC is known as a rare chronic cholestatic liver disease with a prevalence of 6/100.000 in Scandinavia. The disease is progressive and in most cases leads to death, end stage liver disease, or need for liver transplantation. Complications of the disease include bacterial cholangitis, cirrhosis, and development of cholangiocarcinoma (CCA). PSC is most frequently diagnosed among men in early middle age and long-term survival is poor. The pathogenesis of the disease is unknown, but different theories exist and suggest a genetic susceptibility and defects in the innate immune response, triggered by an unknown antigen. PSC is closely associated with inflammatory bowel disease (IBD) and a pathogenetic link between these two diseases is highly possible.

IBD is present in about 80% of patients with PSC; the majority are diagnosed with ulcerative colitis. The IBD in PSC has specific characteristics, such as pancolitis, rectal sparing and backwash ileitis. These patients are also high-risk patients for development of colorectal cancer and dysplasia.

No treatment for PSC is available and although many different anti-inflammatory drugs have been tested, none have been proven to halt disease progression. The bile acid ursodeoxycholic acid (UDCA) initially showed promising results and has been studied as a way to improve survival and also for chemopreventive purposes in PSC but remains controversial. Today the only available treatment option for PSC patients reaching end stage liver disease is a liver transplant. PSC is the leading cause of liver transplantation in Scandinavia today. This is an important group of patients, for whom immunosuppressive treatment and follow-up after transplantation need to be optimized.

1.1.1 Natural history of PSC

Patients are usually diagnosed with PSC in the third or fourth decade of life; mean age at diagnosis is 32-44 years [2-4]. Only about 10% of the patients are diagnosed in childhood. The disease is male predominant; about two-thirds of the patients are men (59-71%). The clinical presentation differs, and many patients (54-70%) are asymptomatic at diagnosis and remain so despite disease progression [3, 5, 6].
In symptomatic patients the disease is characterized by jaundice, upper right quadrant pain, fatigue, pruritus, fever, weight loss and later by signs of end stage liver disease such as encephalopathy and variceal bleedning. PSC is a progressive disease and leads in most patients to development of end stage liver disease, and need for liver transplantation, CCA or death. Reported median transplant-free survival in PSC is between 9 and 18 years [2, 3, 7, 8].
The dreaded complication of CCA is reported to occur in 10-20% of the patients and this malignancy has an exceptionally poor long-term survival [9].

1.1.1.1 Clinical and biochemical features

In the early stages, most patients with PSC are asymptomatic and present with abnormal liver tests as the only sign of liver disease. As the disease advances, symptoms of cholestasis, portal hypertension, fatigue, pruritus, jaundice, weight loss, ascites and abdominal pain frequently develop. Bacterial cholangitis often occurs in patients with PSC. Typically a cholestatic pattern is seen in these patients, with elevated levels of alkaline phosphatase (ALP) as a dominant feature. However, about 10% show normal ALP levels. Also mild to moderate elevations in aminotransferases may be noted; bilirubin levels are often normal, but progressively increase as the disease advances. Several autoantibodies have been detected, including ANCA, ANA, and SMA but none have been shown to be sensitive or specific enough to be used as a screening tool for diagnosis or to be of prognostic value [10].
PSC is usually associated with IBD but is also often associated with autoimmune diseases such as pancreatitis, autoimmune hepatitis, celiac disease and diabetes mellitus [11].

1.1.1.2 Radiological features

Cholangiography remains the standard means for diagnosing PSC. Typical cholangiographic features are usually seen, including irregularities and beading of the intra and/or extrahepatic bile ducts. A meta-analysis has shown that magnetic resonance cholangiography (MRC) is sensitive enough to make the diagnosis; however, endoscopic retrograde cholangiography (ERC) remains the standard diagnostic method [12].

1.1.1.3 Histologic features

PSC is histologically characterized by bile duct proliferation, periportal fibrosis and inflammation and ultimately loss of bile ducts. The fibrosis is often classified into four stages (I-IV) using the Ludwig criteria, where stage IV represents cirrhosis. PSC usually affects both large and small bile ducts but in a subset of patients only the small ducts are involved [13].

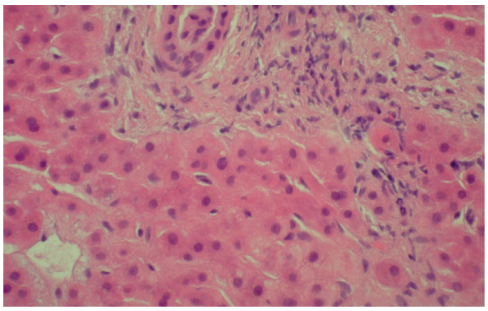


Figure 1. Typical histological appearance of PSC with concentric fibrosis surrounding the bile duct in an onion-skin pattern.

1.1.1.4 Diagnostic criteria

Typically the diagnosis of PSC is based upon the presence of a cholestatic pattern of liver biochemistry, typical cholangiographic findings and the absence of secondary causes of sclerosing cholangitis. (Secondary causes include previous biliary tract surgery, biliary stone disease, and congenital biliary tree abnormalities, cholangiopathy associated with AIDS, portal vein thrombosis or bile duct neoplasm [14].)

1.1.2 Epidemiology

PSC is a rare disease and the true incidence is unknown. Incidence rates between 0 and 1.31 per 100.000 inhabitants per year have been reported. The prevalence varies between different populations and ranges between 0 and 16.2 per 100.000 inhabitants per year. The disease appears to be more common in North America and northern Europe than in Asia, Africa and southern Europe and the differences in prevalence cannot be explained by differences in diagnostic methods alone. In Sweden a prevalence of 16.2 per 100.000 has been reported [15]. The prevalence of IBD in PSC patients has also been noted to be higher in northern European and American populations than southern European and Asian populations [16].

1.1.3 Pathogenesis

The pathogenesis and aetiology of PSC is unknown. Four major theories dominate the conceptions about the pathogenesis of PSC but none of them fully explain the cause of this disease. However, it is generally believed that PSC arises through an immune dysregulation resulting in loss of tolerance, initiated by an unknown antigen in the portal circulation in a genetically susceptible individual.

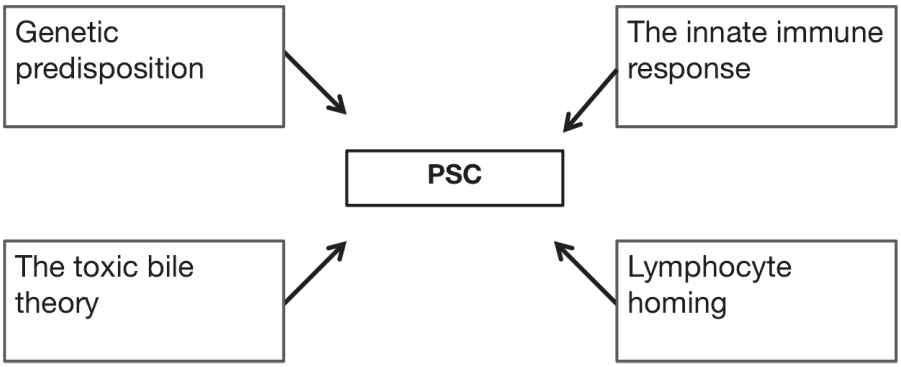


Figure 2. Four theories regarding the pathogenesis of PSC

1.1.3.1 Genetics

Genetic and non-genetic factors that predispose for PSC have been identified but how they increase the risk remains uncertain. PSC, like ulcerative colitis, is a disease associated with non-smoking behaviour; this finding has been published repeatedly [17, 18]. PSC has also been shown to be associated with higher socioeconomic status independent of age, race and gender [19].

The existence of a genetic susceptibility for PSC is generally accepted. A study from Sweden showed that the prevalence of PSC among relatives was 10-fold higher than in the general population [20]. PSC is probably a complex genetic disease meaning that polymorphisms in several genes are involved [21]. A large number of candidate genes have been studied, but with the exception of the results on human leukocyte antigen (HLA) [22-24], most studies have been underpowered.

Recently the first genome wide association study (GWAS) was performed and showed a strong association to HLA but also to a subset of genes involved in bile homeostasis [25].

Considering the strong association between PSC and IBD a shared genetic basis would not be surprising. However, until today most IBD susceptibility genes have failed to show a common genetic link with PSC, with a few exceptions. This lack of a common genetic basis between PSC and UC or Crohn's disease supports the clinical notion that PSC-associated IBD is a unique type of IBD [25-27].

1.1.3.2 The innate immune response and the leaky gut

The close association with IBD suggests that PSC, just like IBD, is not necessarily a classic autoimmune disease. Rather, IBD is the result of an abnormal innate immune response to antigens of the intestinal flora, which activates an adaptive immune response. Several investigators have proposed the activation of the innate immune response as an inciting event of PSC [28].

This theory suggests that an exogenous trigger that enters the portal circulation through an inflamed and permeable gut triggers PSC. As a consequence, inflammatory cells such as macrophages, dendritic cells and natural killer (NK) cells are activated and secrete cytokines and start an inflammatory reaction by activation of NK cells through

IL-12 and recruitment of lymphocytes via TNF-alpha, IL-1b and CXCL8/IL-8. Cholangiocytes are suggested to be the primary targets of the immune attack in PSC.

1.1.3.3 Lymphocyte homing

The observation that PSC often runs a course that is independent from IBD activity has led to the hypothesis that memory T-lymphocytes primed in an inflamed gut persist as long-lived memory cells and can enter the enterohepatic circulation and trigger an inflammation in the liver [29]. Studies that support this theory conclude that livers affected by PSC express the mucosal addressin cell adhesion molecule-1 (MAdCAM-1) which is normally only found in the gut. However, MAdCAM-1 expression is also found in livers affected by other chronic liver diseases such as autoimmune hepatitis, primary biliary cirrhosis and chronic hepatitis C [30, 31]. Thus MAdCAM-1 expression may be a consequence rather than a cause of chronic inflammation. Moreover, IBD does not always precede PSC.

1.1.3.4 The toxic bile theory

Even under normal conditions bile is toxic to cells. Several mechanisms normally protect the bile ducts from injury, including micelle formation and bile flow. Changes in the composition of bile, decreased bile flow, and increased biliary pressure in PSC may all lead to toxic bile formation [31]. Support of the toxic bile theory comes primarily from the multi-drug resistance gene (Mdr2) knockout mouse [32]. Targeted disruption of Mdr2 leads to a PSC-like picture in the bile ducts of these mice, with biliary strictures and onionskin-like fibrosis. However, unlike in human PSC, these mice do not develop IBD.

A clinical observation that supports the toxic bile theory is the finding that colorectal neoplasias and colonic inflammation in PSC often located on the right side, where the concentration of bile is higher.

1.1.4 Prognosis

The long-term prognosis for patients with PSC is highly variable. A large Swedish study of the natural history of PSC reported a median survival of 12 years after diagnosis [3]. A 10-year survival of 65% has been reported in two different studies of PSC [33, 34]. In a more recent study from western Sweden PSC was associated with a four-fold increase in mortality (SMR 4.2) compared with the general population [7]. High age at diagnosis, low albumin and elevated bilirubin have been shown to predispose for a poor outcome [7]. In general, prognostic models are lacking, although a time dependent cox-regression model for prediction of prognosis has been suggested [35]. All models have been shown to be ineffective in predicting the course for an individual patient [14]. Therefore the AASLD practice guidelines do not recommend the use of PSC-specific prognostic models for individual patients.

The risk of hepatobiliary cancers is greatly increased in patients with PSC. In a study by Bergquist et al based on a national cohort of Swedish PSC patients, the standardized incidence rate for hepatobiliary carcinoma was 161, and 37% of all cancers were diagnosed less than one year after PSC diagnosis [9].
A population-based study from Sweden recently confirmed the increased risk: the risk of hepatobiliary cancers was estimated to 177 times that of the general population [7]. About 10-20% of all PSC patients develop CCA [36]. The prognosis of CCA is extremely poor; survival of unresectable CCA is only 12-16 months after onset of symptoms [37].

1.1.5 Treatment

There is no effective medical treatment for PSC. Many different types of medical therapies have been tested, with complete lack of effect. Endoscopic treatment is the optimal way of dealing with dominant strictures of the bile ducts in PSC, but long-term efficacy is questionable. The only effective intervention for end stage disease is liver transplantation, which has a relatively good long-term prognosis. Performing clinical trials of medical therapy in PSC is a big challenge. The disease is rare, has a highly variable course, is slowly progressive, surrogate markers for disease activity are lacking, liver biopsies have a large sampling variability and CCA may develop at any time.

1.1.5.1 Medical treatment

A large number of drugs have been used in trials with PSC patients but none have proven effective. These agents include: corticosteroids, methotrexate, cyclosporine, tacrolimus, colchicine, penicillamine, pentoxifylline, cladribine, nicotine, mycophenolate mofetil, silymarin and bezafibrate [38].

The bile acid ursodeoxycholic acid (UDCA) has been widely used in PSC and is described in detail under section 1.3 of this thesis.

Other agents studied include anti-TNF alpha antibodies that are efficacious in inflammatory bowel disease; however, the results in PSC are disappointing [39, 40]. No improvements were seen in either liver biochemistry or histology. Nonetheless, it is likely that further studies will be performed with other or similar antibodies.

Antibiotics have a potential role in reducing portal antigen influx to the liver and are therefore theoretically an attractive treatment option for PSC. In the largest antibiotic-related randomized controlled trial (RCT) 81 patients with PSC were treated with UDCA with or without metronidazole [41]. In patients treated with both agents for 3 years, an improvement in liver biochemistry was seen but no effect on histology or cholangiography was detected. This finding remains to be confirmed by others.

1.1.5.2 Endoscopy

A dominant stricture in PSC has been defined as a stenosis in the common bile duct that leaves an opening of less than 1.5 mm or <1 mm in a main hepatic duct [42]. The majority of patients with advanced PSC develop dominant strictures and when these lead to complications such as cholangitis, jaundice, pruritus or deranged biochemistry, an ERCP is usually performed.
Endoscopic treatment with balloon dilatation or stenting of dominant strictures may lead to improvements in liver biochemistry and radiology, and alleviate symptoms in some cases [43]. A prospective trial of endoscopy and use of UDCA has suggested a prolonged transplant-free survival, but controlled trials are needed to confirm this [44]. Infective complications and pancreatitis after biliary treatment are common in patients with PSC, and are an important concern [45]. Also re-stenosis after ERCP is common [42].

1.1.5.3 Transplantation

The only effective treatment for PSC is liver transplantation. PSC is the most common indication for liver transplantation in the Nordic countries [46].
The timing of liver transplantation can be difficult in PSC, since the course of the disease is very unpredictable. A decision to enlist a patient for transplantation involves non-disease-specific scoring such as MELD and Child-Pugh, but other indications such as recurrent cholangitis and intolerable pruritus are also accepted.
The general outcome after liver transplantation is good, with a five-year post transplant survival of 80-90% [46]. However, disease recurrence is reported in about 25% of the transplanted PSC patients [47]. A more comprehensive description of liver transplantation and recurrence of PSC is included in section 1.4 of this thesis.

1.2 IBD

Inflammatory bowel diseases are common chronic relapsing inflammatory disorders of the intestines, including ulcerative colitis (UC), Crohn's disease (CD), and indeterminate colitis. The inflammation in UC affects only the colon whereas CD is a granulomatous disease that may affect the entire digestive system and often occurs in patches. The peak age of onset of IBD is between 15 and 30 years.

The diseases have a higher incidence in northern Europe and North America than southern Europe and Asia. However, the incidence in the latter areas seems to be increasing [48]. The change in incidence and the differences in IBD epidemiology are believed to be caused by unknown environmental factors, such as microbial exposure and dietary factors. Incidences of UC range between 0.6-16.5/100.000. CD incidences are generally lower but range between 0-15.6/100.000. The prevalence of the diseases ranges between 6-243/100.000 for UC and 3.6-198/100.000 for CD [49].

The aetiology of IBD is believed to involve an inappropriate immune response that occurs in genetically susceptible individuals and is the result of a complex interaction of environmental factors, microbial factors and the intestinal immune response [50].

The prevalence of PSC in IBD patients is between 2.4-7.5% and the prevalence is higher among patients with extensive colitis. PSC is diagnosed in 2-8% of patients with UC and in 3% of those with Crohn's disease [51-53].
No screening program exists to identify PSC in IBD patients but generally PSC is suspected in patients presenting with a biochemical cholestatic picture with increased levels of alkaline phosphatases.

1.2.1 IBD in PSC

It is now well recognized that PSC is strongly associated with IBD. The prevalence of IBD in patients with PSC in northern Europe and America is reported at 60-80%. However the prevalence varies among different countries and in Japan the rates are as low as 23% [54]. Most patients (80%) present with ulcerative colitis, but 10% are reported to have Crohn's disease and another 10% the indeterminate type [55].
The association with Crohn's disease (regional enteritis) was first recognized by Atkinsson and Caroll in 1964 [56]. Smith and Loe first showed the association between UC and PSC in 1965 [57].
Since then improved knowledge and increased clinical awareness of this association has led to a more active diagnosis of PSC, in patients with UC presenting with a cholestatic picture.

1.2.2 Clinical features

Typical for UC in PSC patients is a pancolitis, with a generally milder course and an increased risk for colorectal dysplasia and cancer [54].
Patients with CD and PSC are less well studied but the IBD has been described as a disease with colonic involvement, and most qualities strongly suggestive of CD such as perianal fistulas, granulomas and small bowel disease are often missing [55].

Loftus et al first described "PSC-IBD" as a unique IBD phenotype in 2005 [55]. According to this concept PSC patients should not be divided into those having UC, CD or IC but rather viewed as having a unique type of IBD: PSC-IBD. In the study by Loftus et al patients with PSC and IBD were compared with a cohort of UC patients.

The PSC patients more frequently presented with extensive colitis (87 vs. 54%), backwash ileitis and rectal sparing. Colorectal surgery was less common in the PSC group, and patients with PSC appeared to have higher risks of colorectal neoplasia.

In general, patients with PSC have a relatively silent inflammatory bowel disease which follows a mild clinical course [58]. Excluding IBD in patients with PSC requires a full colonoscopy with biopsies [59]. Jørgensen et al showed a difference between the macroscopic and microscopic picture in PSC-IBD: in general the inflammatory activity in these patients was low and was not always visible endoscopically, though it could be seen histologically[59].

There appears to be no correlation between the severity of IBD and that of PSC; the diseases seems to run independent courses from each other [51]. IBD usually precedes PSC, but IBD may arise any time during the disease course and even after a liver transplantation *(de novo IBD)* [60, 61].

1.2.3 Risk of colorectal cancer

Patients with UC are considered to have an increased risk of colorectal neoplasia (CRN). Known independent risk factors for colorectal neoplasia in IBD include long disease duration, disease severity, extensive disease, heredity for colorectal cancer and PSC [62]. Colorectal cancers in IBD often arises from flat adenomas but raised lesions, DALM (dysplasia associated lesion or mass), are also present. Epithelium that is considered dysplastic is usually graded into low or high-grade dysplasia. But for dysplasia in IBD the concepts of indefinite probably dysplastic (IPD) or indefinite dysplasia (IND) are also used and describe lesions with borderline dysplasia [63].

Patients with UC and PSC are at a higher risk of developing colorectal dysplasia and carcinoma (CRC) than patients with UC alone [64]. In a meta-analysis, Soetniko et al concluded that the risk is increased about four-fold with an OR of 4.26; (95% CI 2.8-6.48) [65]. The absolute risk seems to increase with disease duration, with a CRC risk of 9%, 31% and 50% after 10, 20 and 25 years in patients with PSC and UC [66]. The risk of CRC in patients with PSC and Crohn's disease is less well studied.
The reason for the increased risk of cancer is unclear but it is speculated that it may be due to a high level of toxic bile acids released in the gut. This theory is supported by the fact that proximal cancers are more common in PSC patients and that early studies of UDCA – which dilutes the toxic bile acids – have shown effects on preventing colorectal cancers in PSC [67-69].
In patients with IBD, the risk of CRC is related to the duration and extent of the disease and this may also be part of the explanation to why PSC patients have an increased risk of cancer since PSC patients may have an asymptomatic pancolonic inflammation that delays both diagnosis and treatment [70].

1.2.4 Treatment of IBD in PSC

Medical treatment of IBD in PSC patients is identical to that of patients with IBD without liver disease. However, the knowledge that patients with PSC have lower inflammatory activity and higher risks of colorectal neoplasia raises certain concerns regarding therapy.

Lower inflammatory activity means less need of medical therapy for IBD. Nevertheless, many patients have 5-aminosalicylates as a long-standing therapy, but the need for intensive immunosuppressive treatment for flares is less common than in IBD without liver disease [71].

Aminosalicylates, such as 5-ASA, have been shown to have chemopreventive properties on colorectal cancer in IBD. However, in PSC no such chemopreventive effects have been shown [68, 72]. Less is known about the chemopreventive effects of long-term use of immunomodulators such as azathioprine [72].

Surveillance colonoscopy is recommended every 1-2 years starting at the time of PSC diagnosis and is encouraged both by the EASL and AASLD guidelines. Surveillance colonoscopies are usually made with random biopsies from 10 standard locations in order to detect flat lesions and additional biopsies from polypoid lesions. A colectomy is generally recommended for PSC patients with dysplasia [72].

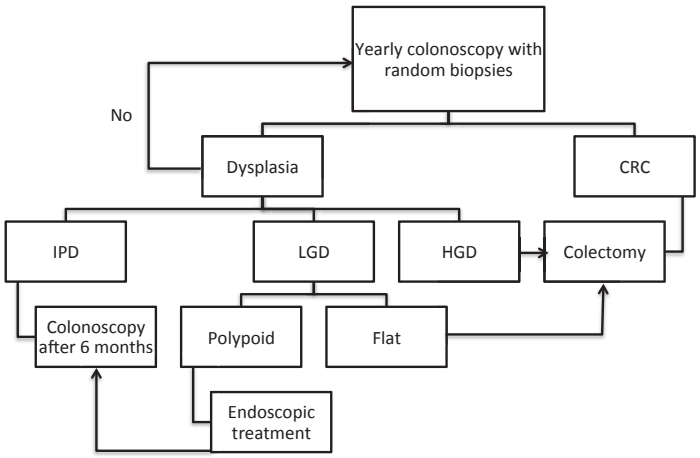


Figure 3: Surveillance for colorectal neoplasia in primary sclerosing cholangitis

Surgery on patients with PSC poses a risk because they may have impaired hepatic function and may decompensate after major bowel surgery.
PSC patients with cirrhosis have a worse prognosis after surgery and have a high rate of postoperative death: 38% compared to 0 in those without cirrhosis, according to one study [73]. Moreover 25% of patients that underwent a proctocolectomy because of UC died or required liver transplantation within 2.5 years after the surgical procedure [74]. Another complication after surgery is that if portal hypertension occurs, peristomal varices may develop in patients with ileal pouch and these can be very difficult to treat [75]. Patients with PSC who have an ileal pouch anal anastomosis after colectomy also have a higher risk of pouchitis compared with patients with UC alone, which suggests a more aggressive disease course after colectomy [76].
These observations suggest that colorectal surgery might seriously affect the course of PSC.

1.3 URSODEOXYCHOLIC ACID

The bile acid ursodeoxycholic acid (UDCA) occurs naturally in low levels in human bile. UDCA intended for use as a drug was initially derived from bear bile, but is now systhesized. UDCA has been widely used in the treatment of PSC and other cholestatic liver diseases such as primary biliary cirrhosis (PBC).
PBC is an autoimmune cholestatic liver disease affecting small bile ducts, and unlike PSC, the disease mainly occurs in middle-aged women. Anti-mitochondrial antigens (AMA) are found in 95% of PBC patients [77].

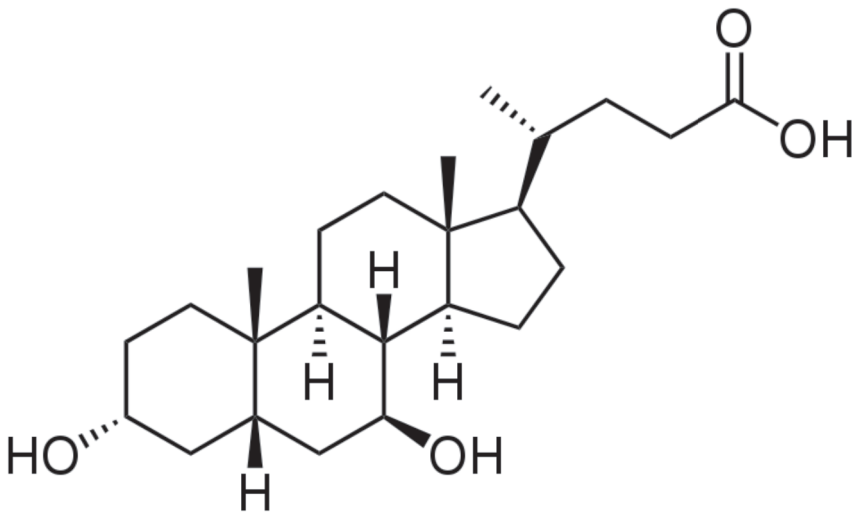


Figure 4. Chemical structure of ursodeoxycholic acid

1.3.1 Indications

UDCA is primarily indicated for use in gallstone disease and in patients with PBC. But the drug also seems to alleviate the effects of cholestasis in pregnancy by reducing pruritus and has also been used in patients with cystic fibrosis as well as in patients with PSC [78, 79].

1.3.2 Mechanisms of action

UDCA is believed to exert a number of effects including cytoprotection, increase of bile flow, protection from apoptosis and immunomodulatory effects. The drug has also been shown to have chemoprotective effects *in vitro* by inhibiting proliferation of tumour cell lines [80].
Cholestatic liver diseases are characterized by defective hepatic excretion of bile acids and an accumulation in serum and tissues; it is believed that UDCA modifies the bile acid pool and thus decreases the levels of secondary, toxic bile acids.

1.3.3 Effect on PBC

UDCA has shown an effect on biochemistry and histology in PBC [81]. However, the effect on long-term survival is less consistent and the latest Cochrane analysis from 2011 does not support a therapeutic benefit of UDCA in PBC. Biochemical response (defined as a reduction of ALP with at least 40%) to the drug is nonetheless associated with better survival and therefore supports treatment with UDCA in patients with PBC [81]. The fact that the rates of liver transplantation for PBC are lower but the prevalence of the disease unchanged supports a benefit of the drug in PBC [82].

1.3.4 Effect on PSC

The first trials on UDCA in PSC were performed in the 1990s and showed promising results with biochemical improvement in the doses of 10-15 mg/kg/day. Since then different doses have been tested, including doses up to 28-30 mg/kg.

1.3.4.1 UDCA in preventing disease progression

The first double blind trial on UDCA in PSC included 105 patients given a dose of 13-15 mg/kg/day. This study showed improvement in biochemistry, but no effect on symptoms, histology, or development of portal hypertension or liver transplantation was seen after 2-5 years of treatment [83].
In this light, higher doses were studied because theoretically higher doses would be needed to enrich the bile and because higher doses might enhance the immunomodulatory effects of the drug. A dose of 20 mg/kg was evaluated in a double blind placebo-controlled trial of 26 patients and showed improvement in liver biochemistry, cholangiographic appearance and histological progression but no effect on survival was detected after 2 years of treatment [84].
The Scandinavian UDCA trial is to date the largest randomized controlled trial (RCT) performed on UDCA in PSC and was a multicentre study that ran over 5 years.

In total 219 patients were included. However, UDCA in the dose of 17-23 mg/kg failed to show anything more than a trend towards increased survival in the UDCA treated group compared to placebo[85]. The drug was considered to be safe and no significant side effects were demonstrated. Even higher doses were tested and pilot studies showed a significant effect on the surrogate marker Mayo Risk score with doses of 25-30 mg/kg. A multicentre trial that included 150 patients given UDCA in a dose of 28-30 mg/kg was conducted, but had to be discontinued early. Patients in the active treatment group, despite improvements in biochemistry, were significantly more likely to reach the primary outcomes of death, liver transplantation and development of varices [86]. The reason for these surprising results is unclear but a possible mechanism might be that a high dose of UDCA leads to increased levels of hepatotoxic lithocolic acid [87].

1.3.4.2 Chemoprevention

A number of studies have suggested that UDCA has chemopreventive effects on the development of colonic neoplasia in patients with IBD and PSC [88, 89]. Theoretically, UDCA may exert its protective effect on colonic mucosa (and on the bile ducts) by reducing the amount of secondary bile acids, by diluting the toxic components of bile. A few small trials have studied the chemopreventive properties of UDCA on colorectal dysplasia and cancer in PSC patients with IBD. In a study by Tung et al, 59 PSC patients with UC undergoing colonoscopic surveillance, the authors found a significantly reduced prevalence of colonic dysplasia in patients taking UDCA [88]. In a follow-up of 52 patients previously enrolled in an RCT, the UDCA-treated patients had a significantly reduced risk of developing CRC or dysplasia [89]. Based on these studies, treatment with UDCA for patients with PSC and longstanding IBD has been recommended. However, other studies have shown no effect on development of neoplasia [90, 91].

Surprisingly, a study from 2010 demonstrated an increased risk of CRC and dysplasia in patients treated with very high doses of UDCA (28-30 mg/kg) [92]. In this study treatment with UDCA had a Hazard Ratio of 4.44 (however very wide CI 1.3-20.1). The American Association for the Study of Liver Diseases (AASLD) discourages the use of UDCA in PSC based on the negative outcomes in UDCA-treated patients in the study that employed UDCA at a very high dose [14]. Long-term data and large prospective randomized trials regarding UDCA's chemopreventive effect (in low or moderate doses) on the colorectal mucosa in PSC are lacking, and UDCA remains controversial as a chemopreventive agent.

1.4 LIVER TRANSPLANTATION IN PSC

Today the only possibly curative treatment of PSC is liver transplantation.

1.4.1 History

The first human liver transplantation was performed in 1963 by Thomas Starzl. The results from the first transplantations were disappointing however with very poor survival.

With the introduction of cyclosporine in the immunosuppressive treatment, liver transplantation became more successful and transplantation programs expanded during the 1980s. In Sweden the first liver transplantation was performed in 1984 at Huddinge Hospital and the first transplantation on a patient with PSC was done one year later. Today PSC is the most common indication for liver transplantation in the Nordic countries, representing about 20% of all indications. To date a total of 717 PSC patients have been transplanted in the Nordic countries (www.scandiatransplant.org). In North America PSC represents the fifth most important cause of liver transplantation. The differences between Scandinavia and North America may be explained by differences in the prevalence of hepatitis C.
Indications for liver transplantation for PSC are wider today than previously and include intractable pruritus and early CCA in some patients.

1.4.2 Drugs

Conventional immunosuppression after liver transplantation includes triple therapy with corticosteroids, a calcineurin inhibitor (such as cyclosporine or tacrolimus) and azathioprin or mycophenolate mofetil. Cyclosporine was used for liver transplanted patients primarily until 1995, when it was superseded in most patients by tacromlimus. The immunosupressive regimen after OLT for PSC is similar to that after transplantation for other liver diseases. There are no controlled studies evaluating different immunosuppressive treatments after OLT in PSC [46]. However, most transplantation centres recommend lifelong corticosteroid treatment for PSC patients, because they are at higher risk of rejection than patients with other conditions such as hepatitis C or alcoholic liver disease.

1.4.3 Surgery and the prognosis after liver transplantation

The timing of transplantation may be extremely difficult in PSC, due to the unpredictability of the disease and the risk of developing hepatobiliary carcinoma, which is an absolute counterindication for OLT in most cases. A prophylactic OLT for PSC is of course not an option due to the high morbidity and mortality after a liver transplantation and to the lack of organs.

Most surgeons perform a hepatico-jejunostomy with a Roux-en-Y loop in PSC patients undergoing OLT, due to a hypothetical risk of malignancy and stricturing of the bile ducts if an end-to-end anastomosis is performed. The survival of both graft and patient is improved in PSC patients receiving a Roux-en-Y loop as compared to an end-to-end anastomosis [93].

PSC patients have an increased rate of both corticosteroid sensitive and corticosteroid non-sensitive rejections after OLT, compared with patients with patients with end stage liver disease due to alcohol cirrhosis and hepatitis B or C [46].

The long-term survival of PSC patients undergoing liver transplantation is good and comparable with patients with other chronic liver diseases such as PBC. Moreover, survival of patients transplanted for PSC is generally better than for patients undergoing OLT for hepatitis C.

The five-year survival in the Nordic countries is reaching 90% for PSC patients (www.scandiatransplant.org). However, the threat of recurrent disease in PSC patients remains, and recurrence might have a greater impact on graft and patient survival than was initially believed.

1.4.3.1 Recurrent PSC

Recent reports have demonstrated that about 1/5 of all liver transplanted PSC patients develop recurrent disease after transplantation. Lerut et al published the first article describing recurrence of PSC in 1988 [94]. Since then numerous reports have described this phenomenon that is now regarded as a diagnostic entity. In a review by Fosby et al the rate of recurrence differs between studies (range 5-59%) probably due to differences in diagnosis, the length of follow-up and study design [47]. In the largest study of 236 patients, 23.5% suffered recurrence after a median time lapse of 4.6 years [95].

Many potential risk factors have been proposed and the one most frequently studied is the connection with IBD. In a study by Vera et al, male sex and an intact colon were the strongest risk factors for recurrence of PSC [96]. Another interesting observation is that the rate of recurrence is increased in patients with a living related donor, with recurrence rates over 50% [47].

1.4.3.2 IBD after OLT: IBD activity and risk of colorectal cancer

The immunosuppression used after a liver transplantation is in many cases the same as potential therapeutic options for IBD, such as cyclosporine, corticosteroids, tacrolimus, MMF and azathioprine. Conflicting reports have been published regarding the activity of IBD after OLT, where some authors suggest a more active course of IBD whereas others an unchanged pattern of activity [97-99]. No specific immunosuppressive regimen for PSC patients with IBD has been established, but prolonged use of corticosteroids has been suggested to reduce the risk of IBD flares.

There has been concern that the immunosuppressive treatment given after OLT confers an additional risk of colorectal malignancies in PSC patients with IBD and it has been suggested that liver transplanted PSC-IBD patients run a higher risk of colorectal malignancies than non-transplanted ones. On the other hand, OLT did not have any influence on the incidence of colorectal cancer in a cohort of 192 PSC-IBD cases [100]. The impact of OLT on the risk of colorectal malignancies in PSC-IBD post transplant remains unsettled.

2 AIMS

The general aim of this thesis was to study the characteristics of inflammatory bowel disease and the risk of colorectal neoplasia before and after liver transplantation in patients with primary sclerosing cholangitis.

We also aimed to study the effects of ursodeoxycholic acid both on survival and development of colorectal neoplasia in patients with PSC.

We specifically aimed to answer the following questions:

1. What are the clinical characteristics of Crohn's disease in PSC? (Paper I)
2. Is the risk of colorectal cancer increased in patients with PSC and Crohn's disease (as well as in PSC patients with ulcerative colitis)? (Paper I)
3. Is the clinical course of IBD in PSC patients changed after a liver transplantation? (Paper II)
4. Does the risk of colorectal cancer further increase after liver transplantation? (Paper III)
5. Does long-term treatment with UDCA protect against colorectal cancer and dysplasia? (Paper IV)
6. Does treatment with UDCA improve long-term survival in patients with PSC? (Paper V)
7. Is the clinical outcome different between biochemical responders and non-responders to UDCA? (Paper V)

3 MATERIALS AND METHODS

The five studies included in this thesis are based upon data from three different cohorts of PSC patients. All studies were ethically approved according to the regional ethics committee of Stockholm. Paper II and III were conducted in cooperation with the Nordic liver transplant group (NLTG).

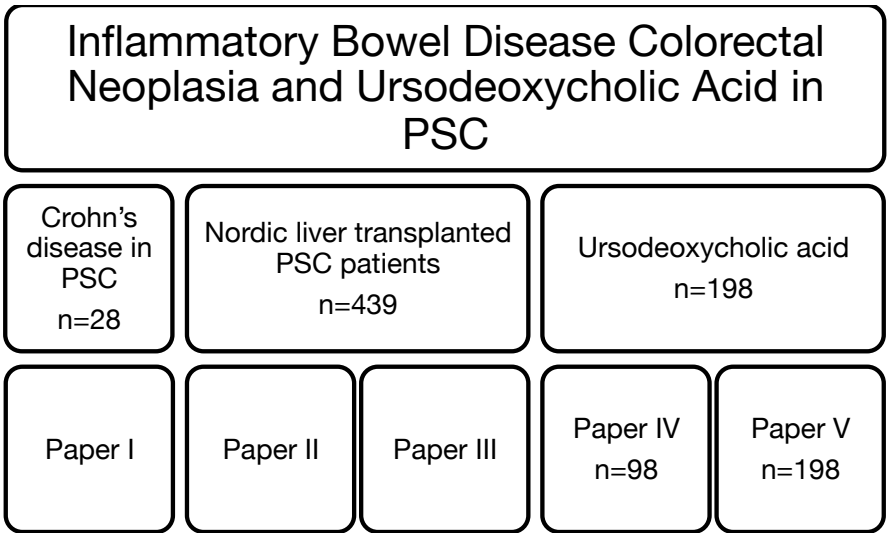


Figure 5. Cohorts and papers included in this thesis

3.1 COHORT I (PAPER I) CROHN'S DISEASE IN PSC

3.1.1 Patients

The study population was recruited from the PSC cohort at Karolinska University Hospital. This database was constructed in 2003 and includes all PSC patients treated at Karolinska University Hospital at Huddinge. By 2006 the registry held 290 patients and all patients with concomitant diagnosis of Crohn's disease were included. In total 28 patients with CD and PSC were registered in the database and included in the study.

3.1.2 Matching

The study is a matched cohort study. Every patient with PSC was matched to two controls with colorectal Crohn's disease without liver disease. The controls were identified from a large and well-defined cohort of patients with Crohn's disease in Stockholm [101]. The matching criteria used were: age (± 2 years) and year of onset of IBD (± 2 years). The matching was used in order to minimize confounders and to achieve similar duration, treatment and cancer surveillance patterns in the two groups.

3.1.3 Data collection

Data were collected in a specific protocol and obtained by manually scrutinizing medical records, the PSC database, and histological review. Collected clinical data included: date of diagnosis of PSC, distribution of PSC, liver transplantation, distribution of IBD, colorectal surgery performed, extraintestinal manifestations of IBD, family history of IBD and liver disease, bowel resection, fistulas and perianal disease and bowel strictures. All medical treatment for PSC and IBD was recorded, including dose and duration of 5-ASA, systemic steroids, azathioprine and UDCA. We collected data from all colonoscopies performed from the onset of IBD and recorded the endoscopic features in both groups. Extension of disease, rectal sparing, fistulas, strictures, segmental inflammation, aphtous ulcers, or large serpiginous longitudinal or deep ulcers were also recorded on the basis of endoscopic reports. Endoscopic reports were also used to collect information on macroscopic cancer or suspect dysplasia. Histological reports from all colonoscopies and colonic resections were scrutinized and categorized for grade of dysplasia and histological characteristics. Colonic biopsies from patients with PSC were reviewed by a pathologist specialized in gastrointestinal diseases (Åke Öst).

3.1.4 Definitions

The histological findings of dysplasia were categorized as indefinite probably dysplastic (IPD), low grade of dysplasia (LGD) or high grade of dysplasia (HGD) [102]. Location of colorectal dysplasia or cancer in the transverse colon or more proximally was classified as proximal and location in the left flexure or more distally was classified as distal. The diagnosis of CD was based on typical clinical, radiological, endoscopic and histological findings according to the Lennard-Jones criteria [103]. The onset of CD was defined as the time of first presentation of symptoms and clinical signs of CD. We excluded patients who did not undergo at least two colonoscopies with colonic biopsies or whose medical records could not be retrieved. The number of years at risk of dysplasia was defined as from the time of onset of CD until death or colectomy, until cancer or dysplasia was detected, or until the end of the study. For patients whose diagnosis changed from UC to CD, the onset of disease was defined as first date of UC.

3.1.4.1 Lennard-Jones criteria

There is no “gold standard” for diagnosing Crohn’s disease, but the European Crohn’s and Colitis Organization (ECCO) still refers to the Lennard-Jones criteria, published in 1989. Macro- and microscopic features compromise the Lennard-Jones criteria for Crohn’s disease. According to these criteria, the presence of any number of the features can define the disease, but use of three features is suggested. Granuloma, when present, is regarded as diagnostic and is therefore given greater weight than other features.

Table 1. The Lennard-Jones anatomic criteria for the diagnosis of Crohn's disease

	Clinical/Endoscopy	X-ray	Biopsy	Specimen
Mouth to anus				
Upper gut	+	+	+	+
Anus	+		+	+
Discontinous	+	+	+	+
Transmural				
Fissure		+		+
Abscess	+	+		+
Fistula	+	+		+
Fibrosis				
Stenosis	+	+		+
Lymphoid				
Ulcers			+	+
Aggregates			+	+
Mucin				
Retention			+	
Granuloma			✚	✚

Crohn's = + + + or + ✚ (exclude infection or ischemia)

3.1.5 Statistical analysis

Differences between PSC patients and controls were calculated using chi-square test or unpaired 2-tailed student t-test. Fisher's exact test was used when appropriate. P-values less than 0.05 were considered significant. Kaplan-Meier survival curves with log rank significance test were used to compare time to CRC or dysplasia between the two groups. Dysplasia and CRC were used as terminal events and colectomy and death were censoring variables.

3.2 COHORT II (PAPER II AND III) THE NORDIC LIVER TRANSPLANTED PSC PATIENTS

3.2.1 Patients

Paper II and III are multicentre cohort studies. The Nordic liver transplant registry (NLTR) was used to identify a total of 461 PSC patients undergoing liver transplantation from November 1984 through December 2006. The NLTR was initiated in 1988 and covers all patients on the waiting list for liver transplantation from 1982 until today.
Twenty-two patients were excluded from the studies (the diagnosis could not be confirmed or patients were lost to follow-up). Among the remaining 439 patients, 122 underwent OLT in Göteborg, 95 in Oslo, 93 in Stockholm/Uppsala, 83 in Helsinki and 46 in Copenhagen. The diagnosis of PSC was made according to accepted criteria with typical bile duct irregularities on cholangiography [104].

The diagnosis of IBD was based on conventional clinical, endoscopic and histopathological criteria [51]. Transplantation was performed in a standard fashion with no living donors and with a Roux-en-Y choledochojejunostomy performed in the majority of patients (375/439). All patients were regularly followed at the transplant centres.

3.2.2 Data collection

Physicians at each transplant centre reviewed the medical records of the patients. Data were retrieved according to a common protocol that included the time span from diagnosis of IBD until the last clinical follow-up. When necessary, medical records were retrieved from the referring hospitals. Date, type and cause of colectomy were also recorded. Detailed records of the medical therapy given for PSC and IBD and for the immunosuppressive therapy after OLT were obtained. Acute cellular rejection treated with steroids, antithymocyte globulin (ATG) or muromonab-CD3 (OKT3) and treated cytomegalovirus (CMV) infections during the first six months post transplantation were recorded. We also recorded the HLA status of the recipient.

3.2.3 Definitions

3.2.3.1 Paper II

The IBD activity was measured in different ways 1) Macroscopic findings at endoscopy 2) IBD relapses and 3) IBD activity curves.

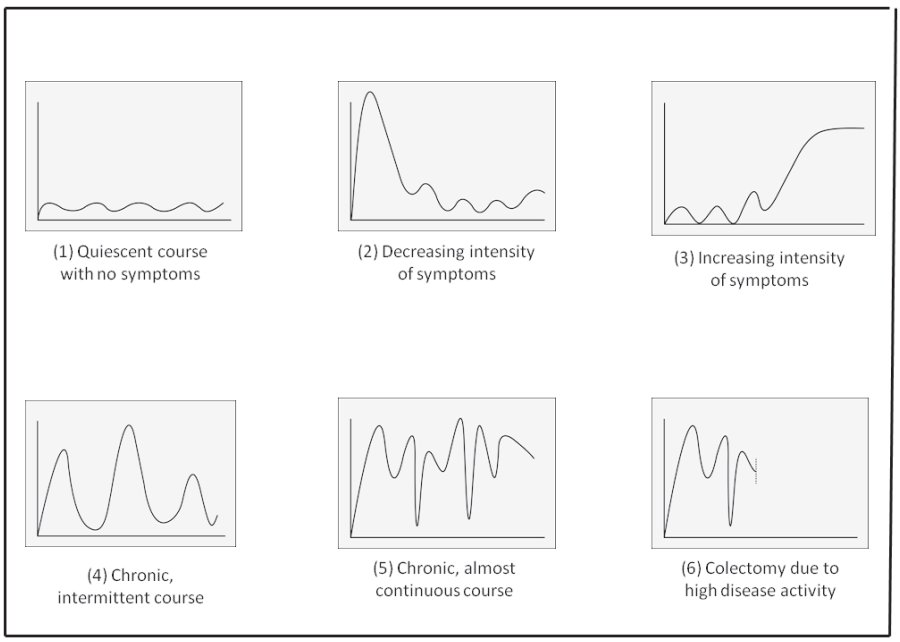


Figure 6. IBD activity curves

Macroscopic findings at endoscopy were recorded at diagnosis, at the last colonoscopy performed before OLT, the first after OLT and at the examination closest in time to the last clinical follow-up. The colonic inflammation was graded as normal, mild, moderate or severe [105]. Of the two endoscopies after OLT, the one with the most severe inflammation was selected for comparison with the pre OLT investigation. The diagnosis of *de novo* IBD was based on macroscopic and/or microscopic findings at endoscopy post OLT and required a normal colonoscopy before surgery.

The frequency of IBD relapses during the last three years before and the first three years after OLT was recorded. Patients with a history of IBD less than two years before and/or less than two years of follow-up were excluded from this sub-study. A relapse was considered if one or more of the following events were recorded: (a) an increase in IBD-related symptoms leading to consultation of a specialist, (b) initiation or increase in dose of IBD medication, (c) increase in stool frequency, (d) macroscopic faecal blood related to IBD, (e) worsening or verified IBD-related macroscopic findings at endoscopy and (f) colectomy for high IBD disease activity.
IBD activity curves were used to record the clinical activity during the total course of IBD in each patient. The curves are based on a modification of previously reported disease course patterns [106].

3.2.3.2 Paper III

Histological reports were used to retrieve findings of colorectal neoplasia during the pre and post OLT disease course. Colorectal neoplasia was categorized as LGD, recurrent-LGD, DALM, HGD or carcinoma. In each patient, colorectal neoplasia was categorized as the most advanced lesion ever noted.

3.2.3.3 Medical therapy

For drugs with a potential effect on colorectal carcinogenesis (aminosalicylates, azathioprine, ursodeoxycholic acid) a patient was defined as using a certain medication if he/she took the drug for a minimum of one year during the last two years before and/or the first two years after the transplantation. For the immunosuppressives used after OLT (tacrolimus, cyclosporine A, mycophenolate mofetil), an intake for a minimum of three months during the first two years post OLT was defined as using a given medication. In paper II a patient was defined as using a certain immunosuppressant at a minimum of three months during the first six months after transplantation.

3.2.4 Statistical analyses

Data were described with proportions for categorical variables and median with range for continuous variables. Crude associations were assessed with Chi-square test or Fishers exact when appropriate. Mann-Whitney test was used for comparisons between groups with respect to continuous variables. Patient survival was calculated using the Kaplan-Meier method, and survival times were compared with the log-rank test. For comparison of observer-dependent variables such as the frequency of colonic inflammation pre and post OLT, McNemar's test was used.

3.2.4.1 Competing risk regression analysis

Survival analysis involves measuring the time from a specific origin until an event of interest occurs. The data consist of patients that experience an event and censored individuals (patients who do not reach the event of interest). However, in most studies some patients experience an event other than the one of interest. This is usually termed a competing event and these patients are censored at the time the competing event occurs. In many cases the Kaplan-Meier method or the Cox proportional hazards model is used; in these two approaches the censoring is assumed to be non-informative, i.e., the censoring is assumed to be independent from the mechanism causing the patient to be censored. However, in many cases the patient may experience an event (other than the one of interest) that actually alters the probability of experiencing the event of interest, in which case the censoring is called informative. In this setting, death or colectomy for other causes were such events, and have to be considered informative censoring. Such events are often termed *competing risk events*.

Patients who died or had undergone colectomy for other reasons could not experience neoplasia: treating them as censored would lead to overestimation of the cumulative risk of neoplasia.

The Kaplan-Meier method or the Cox proportional hazards model cannot always accurately estimate the cumulative incidence when competing events are censored. Cumulative incidences are usually overestimated with Kaplan-Meier or the regular Cox model especially in the case of informative censoring, long follow-up times and many competing events [107]. In this case, in order to accurately assess the risks, we have used a modified Cox proportional hazards model or the competing risk regression approach, developed by Fine and Gray in 1999.

3.2.4.2 Competing risks in Paper II

The cumulative risks of colectomy for refractory IBD before and after OLT were estimated using competing risk regression analysis. Colectomy for refractory IBD was defined as the main event of interest and colectomy due to other reasons and death were competing events.

The severity of IBD activity pre and post OLT in each patient and the relapse rate before and after OLT were compared using Wilcoxon Signed Ranks Test for paired data. The effect of medication and other factors on the course of IBD post OLT was studied using a Cox proportional hazards model with univariate and multivariate analyses, stratified by the different transplant centres.

3.2.4.3 Competing risks in Paper III

The cumulative risks of colorectal neoplasia were estimated using competing risk regression analysis. We intended to compare the risk before and after OLT, so that patients who did not experience neoplasia before OLT were censored at the time of OLT with a follow-up that started at the time of diagnosis.

The diagnosis of neoplasia was defined as the main event and death and colectomy for other reasons than neoplasia were competing events. To investigate the effect of IBD duration on the risk of neoplasia after OLT, a competing risk regression model was fitted, with neoplasia being the main event, and death and colectomy for other purposes as the competing events. In this model IBD duration was treated as a categorical variable.

3.3 COHORT III (PAPER IV AND V) THE SCANDINAVIAN UDCA STUDY

Between 1996 and 2001 a randomized, double blind, placebo controlled Scandinavian trial was conducted that aimed to investigate the effect of UDCA on survival without liver transplantation. In total 110 patients were randomized to UDCA and 109 to placebo. Twenty-one patients were excluded because they did not come to any follow-up appointments or never took the capsules. So 97 treated and 101 placebo patients remained and were included in the trial. Papers IV and V are based on the Scandinavian UDCA trial cohort.

3.3.1 Patients

In paper IV all patients from the Scandinavian UDCA trial that had a concomitant IBD were included, n=168. After exclusion of 50 patients who had undergone proctocolectomy before study entry (20 due to cancer or dysplasia), five patients who developed colorectal cancer or dysplasia before or within 6 months of study entry and 15 patients who were not included in surveillance programmes, 98 patients remained. Of these patients 48 had been allocated to UDCA and 50 to placebo. In 2009, for the last follow-up of the cohort, we were able to trace 77 patients. In paper V all 198 patients were studied; 28 patients were missing for the follow-up in 2009/2010.

3.3.2 Data collection

The investigators from the original trial collected the data using medical records, and for some data the original trial database was used. If the original trial investigator was unavailable, Lina Lindström collected data. Data collected included data from all colonoscopies performed, data on colorectal surgery, medical treatment after the end of the trial, survival data, prevalence of colorectal cancer or dysplasia, transplantation, and development of CCA during and after the trial.

3.3.3 Definitions

Colorectal neoplasia was categorized as LGD, HGD and CRC and these were used as primary endpoints in paper IV. Lesions classified as indefinite dysplasia (IND) were not taken into account.
In Paper V, the endpoints considered were death, liver transplantation or diagnosis of cholangiocarcinoma. Patients who had a reduction of ALP levels of at least 40% after 1 year in the trial as well as patients with consistently normal levels were categorized as responders [81].

3.3.4 Statistics

Descriptive statistics were used to characterize the data. Cancer-free survival was assessed using the Kaplan-Meier model with a log rank test; colectomy and death were used as censoring variables (Paper IV). The endpoint-free survival in paper V was assessed using the Kaplan-Meier method, with log rank test, and survival was compared between treated and untreated patients as well as between "ALP responders" and non-responders.

4 RESULTS

4.1 INCREASED RISK OF COLORECTAL CANCER AND DYSPLASIA IN PATIENTS WITH CROHN'S COLITIS AND PRIMARY SCLEROSING CHOLANGITIS (PAPER I)

4.1.1 Patient characteristics

Twenty-eight CD patients with PSC and 46 CD patients without liver disease were studied. Median age at CD diagnosis was 26 years in PSC patients (range, 4-57) and 26 years (range 5-56) in controls. Sixty-one percent of the PSC patients were male. Median number of years at risk for dysplasia was 12 in PSC patients vs. 10 in the control group. Patients and controls were examined with colonoscopy with similar frequency: mean 5 vs. 4 colonoscopies during the observation period. Five of the PSC patients died during follow-up vs. three of the controls. Four (14%) of the PSC patients were smokers (current or former) and in the control population 21 patients (46%) smoked ($P = 0.002$).

4.1.2 Clinical and endoscopic characteristics of Crohn's disease

The characteristics of CD in the two groups are described in table 1. The endoscopic findings were similar in the two groups: most patients had a discontinuous inflammation. Fistulas and abscesses were rare in the PSC group and occurred in only 1/28 patients. Bowel strictures and bowel surgery were also uncommon in PSC patients as compared to patients with CD alone. One (4%) of the PSC patients developed small bowel involvement compared with 10 (22%) of the matched controls. The proportion receiving medical treatment with 5-ASA did not differ between patients and controls: 82% vs. 87% respectively.

Table 2. Clinical features, treatment and outcomes in patients with Crohn's disease and PSC compared with controls with colorectal Crohn's disease and no liver disease.

	PSC-CD (n=28)	CD (n=46)	P-value
Bowel surgery (n (%))	5 (18)	21 (46)	0.01
Rectal sparing at diagnosis (n (%))	9 (32)	11 (24)	NS
Extraintestinal IBD manifestations (n (%))	8 (29)	22 (48)	NS
Fistulas/and or abscess (n (%))	1 (4)	15 (33)	0.003
Bowel strictures (n (%))	2 (7)	13 (28)	0.03
Treatment with 5-ASA ≥ 2 years (n (%))	23 (82)	40 (87)	NS
UDCA treatment (n (%))	21 (75)	0 (0)	NA
Endoscopic discontinuous inflammation (n (%))	22 (79)	38 (83)	NS
Endoscopic aphtous ulcers (n (%))	15 (54)	27 (59)	NS

NS=not significant, NA=not applicable

4.1.3 Histological evaluation

Colonic biopsies from all PSC patients were re-reviewed. In 11 of 28 (39%) of the patients the CD diagnosis could be confirmed histologically. There were no significant differences between the two groups regarding histological features. Granulomas occurred in 29% of the PSC patients vs. 43% of the controls, ($P = 0.19$).

4.1.4 Colorectal carcinoma and dysplasia

There was a significant difference in cancer and dysplasia-free survival between the two groups (P = 0.009, log rank test). Three of the PSC patients developed CRC, and one of them died; no patients in the control group developed CRC. The crude frequencies of CRC or dysplasia were 9/28 patients in the PSC group vs. 3/46 in controls, with an odds ratio of 6.78; 95% CI (1.65-27.9).

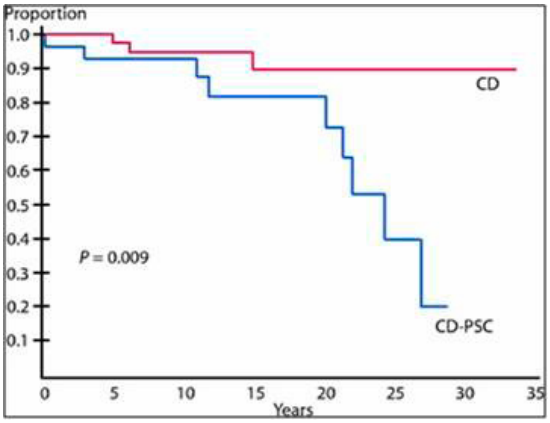


Figure 7. Colorectal cancer and dysplasia-free survival in patients with Crohn's colitis associated with PSC (CD-PSC) and in patients with Crohn's colitis without PSC (CD)

4.2 CHOICE OF IMMUNOSUPPRESSION AFTER LIVER TRANSPLANTATION FOR PRIMARY SCLEROSING CHOLANGITIS INFLUENCES ACTIVITY OF CONCOMITANT INFLAMMATORY BOWEL DISEASE (PAPER II)

4.2.1 Study population

Of the 439 transplanted PSC patients, 353 (80%) had a concomitant diagnosis of IBD at the time of OLT. The majority of patients (70%) were male, and median age at PSC diagnosis was 36 years (range 6-70). Median age at liver transplantation was 44.5 years among all patients and 44 years among those with IBD. The type of IBD was in 307 patients (87%) ulcerative colitis, in 32 (9%) Crohn's disease and in 15 patients (4%) IBD-unclassified. Median duration of IBD at the time of OLT was 15 years, median follow-up after OLT was 5 years (range 0-21). In total 63 patients had more than one OLT. Eleven patients developed IBD after OLT. Of the 270 patients with an intact colon at OLT the patients with less than 6 months post OLT follow-up (n=23) were excluded as well as those who lacked a colonoscopy after OLT, leaving 218 patients to be studied.

4.2.2 Macroscopic inflammation

We found macroscopic inflammation in 124 patients (57%) before OLT and in 153 patients (70%) after OLT, ($P < 0.001$). The frequency of active colitis after OLT was significantly higher among patients with pre OLT inflammation ($P < 0.001$). Also the number of colectomies due to high IBD activity was significantly higher among the patients with pre OLT inflammation compared with those without: 19 of 125 vs. 1 of 94 ($P = 0.001$).

When we compared macroscopic inflammatory changes before and after OLT, the IBD activity was alleviated in 37 (17%) patients, remained unchanged in 93 (43%) and worsened in 88 (40%). No difference was seen in survival between the group with and without IBD relapse ($P = 0.55$).

4.2.3 Relapse of IBD

A significantly higher number of patients had experienced one or more episodes of relapse during the first three years after OLT compared with the last three years before OLT 77 (41%) vs. 53 (28%) ($P = 0.01$). There was also a significant increase in the relapse rate (relapses/person years) after as compared to before OLT ($P < 0.001$).

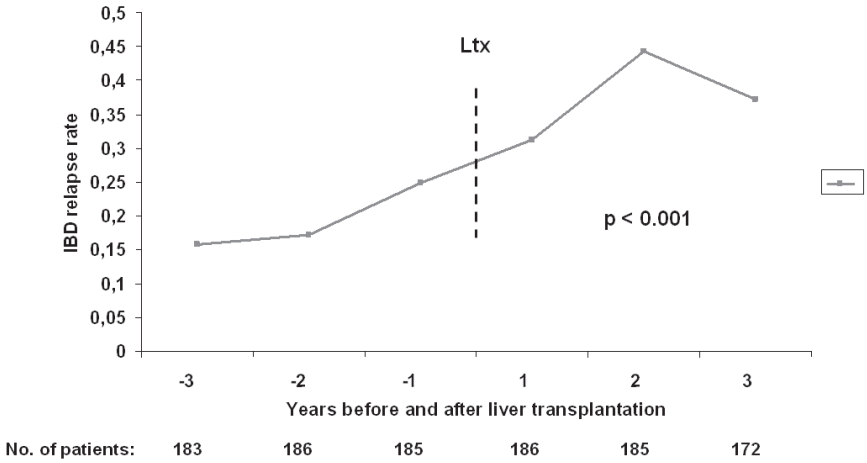


Figure 8. Number of relapses per person-year during the last three years before and the first three years after liver transplantation

4.2.4 IBD activity curves

The clinical activity measured with the IBD activity curves of the total course of IBD before and after OLT revealed that a significantly larger number of patients had experienced clinical IBD activity post OLT compared to pre transplant (96 (44%) vs. 50 (23%), $P < 0.001$). However, the majority of patients had a quiescent course with no symptoms.

4.2.5 Cumulative risk of colectomy due to active disease

In total 127 patients (35%), all with concomitant IBD, had undergone colectomy. Ninety-four colectomies had been done before (52 active disease, 24 neoplasia, 13 both, 5 other causes) and 33 after OLT (14 active disease, 10 neoplasia, 6 both, 3 other causes).

The cumulative risk of colectomy before OLT was 1.5%, 4.8%, 6.2% and 23% at 1, 3, 5, 10 and 20 years after IBD diagnosis. The corresponding risk after transplant was 0.4%, 2.7%, 5.4% and 8.3% at 1, 3, 5 and 10 years after OLT.
The cumulative risk of colectomy due to active IBD in the group of patients still at risk after OLT (n=259, 20 endpoints) was increased compared to the corresponding risk before OLT (n=353, 65 endpoints) but without reaching statistical significance: Hazard ratio 1.4; 95% CI (0.4-1.2, p=0.22).

4.2.6 Risk factors for increased IBD activity

All patients used 5 mg of prednisone as long-term maintenance treatment after OLT. As additional immunosuppression 142 patients used tacrolimus (71 alone and 65 in combination with MMF, 6 in combination with other compounds) and 74 patients used cyclosporine A (CsA) (20 alone, 50 in combination with azathioprine and 4 in combination with other compounds).
A univariate analysis identified age<20 years at diagnosis of IBD, use of tacrolimus and dual therapy with tacrolimus and MMF as significant risk factors for worsening of IBD, whereas dual treatment with CsA and azathioprine showed a significant protective effect. All significant factors in the univariate analysis except for tacrolimus remained significant in the multivariate analysis.

4.3 COLORECTAL NEOPLASIA IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS UNDERGOING LIVER TRANSPLANTATION: A NORDIC MULTICENTER STUDY (PAPER III)

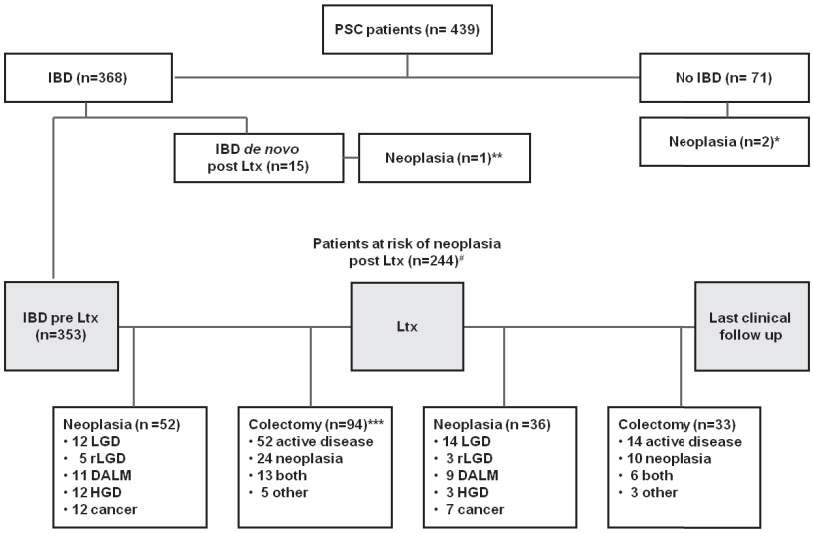
4.3.1 Study population

The study population is similar to that of paper II and consists of 439 PSC patients liver transplanted for PSC; 368 (80%) had concomitant IBD. Among the patients who were still at risk for neoplasia from the time of OLT (n=244), 210 had undergone one or more colonoscopies post OLT. A pre OLT colonoscopy was performed in 94% of the patients diagnosed with neoplasia post OLT, at median of 6.5 months before OLT. The two patients who lacked a pre OLT colonoscopy had both previously undergone colonoscopy after OLT without findings of neoplasia.

4.3.2 Colorectal neoplasia and colectomy in the total cohort of PSC patients

In total 91 (25%) of the 368 patients with concomitant IBD developed colorectal neoplasia during the study period. Among these were two patients who had undergone subtotal colectomy with an ileorectal anastomosis (IRA) due to high disease activity and later developed neoplasia in the remaining rectum. However, these cases were censored along with the other cases who had an IRA at the time of colectomy and the two neoplasias were not included in the risk analysis.
Two patients without IBD developed neoplasia after OLT and one patient with de novo IBD developed rectal LGD.

The median age at diagnosis of colorectal neoplasia was 42 years (21-69), and the neoplasia was diagnosed at median 18 (0-42) years after the diagnosis of IBD.
A remaining 88 (25%) of the 353 patients with IBD before OLT developed colorectal neoplasia, including 52 (59%) before and 36 (41%) after the transplant.
Fifty-four of the neoplasias were classified as DALM, HGD or cancer. Median time from OLT to neoplasia was 5.6 (0.3-18) years.
Fifty-seven (68%) of the 84 neoplasias with known location involved the proximal colon. Colectomy was carried out in 127 (36%) of the 353 patients.



*1 LGD in the rectum 11 years post Ltx, 1 carcinoma in the rectum 9 years post Ltx
**1 LGD in the rectum 16 years post Ltx
***1 patient with IRA 7 years pre Ltx developed a DALM in the rectum 7 years post Ltx, 1 patient with IRA 1 year post Ltx developed a rectal carcinoma 15 years post Ltx
patients with neoplasia (n=52) and colectomy for non-neoplastic reasons (n=57) pre Ltx are excluded
PSC, primary sclerosing cholangitis; IBD, inflammatory bowel disease; Ltx, liver transplantation; LGD, low grade dysplasia; rLGD, recurrent low grade dysplasia; DALM, dysplasia-associated lesion or mass; HGD, high grade dysplasia; IRA, ileorectal anastomosis

Figure 9. Overview of PSC patients (n=439) included in the study.

4.3.3 Patient survival

At the end of the study 113 patients (26%) had died. Crude post OLT survival calculated using the Kaplan-Meier method was 88.1 after 1 year, 79.1% after 5 years and 70.7% at 10 years. The post OLT survival did not differ between patients with and without IBD.

4.3.4 Cumulative risk of colorectal neoplasia in PSC patients with IBD

The cumulative risk of any kind of colorectal neoplasia in the 368 patients with IBD calculated from the time of IBD diagnosis was 3.3%, 6.4%, 16.7% and 25% after 5, 10,

20 and 25 years. However, the risk of colorectal carcinoma was only 0.6%, 1.8% and 3.3% after 5, 10 and 20 years.
The cumulative risk of any type of neoplasia in the group of patients still at risk after OLT (n=244, 36 cases of neoplasia) was higher than the corresponding number before OLT (353, 52 cases) (HR: 1.9: 95% CI 1.3-2.9, P = 0.002) (Fig. 9A).
Using DALM, HGD and carcinoma as a combined endpoint the risk of neoplasia post OLT was also increased but did not reach statistical significance. (HR 1.55, 95% CI 0.89-2.68, P= 0.121) (Fig. 9B).

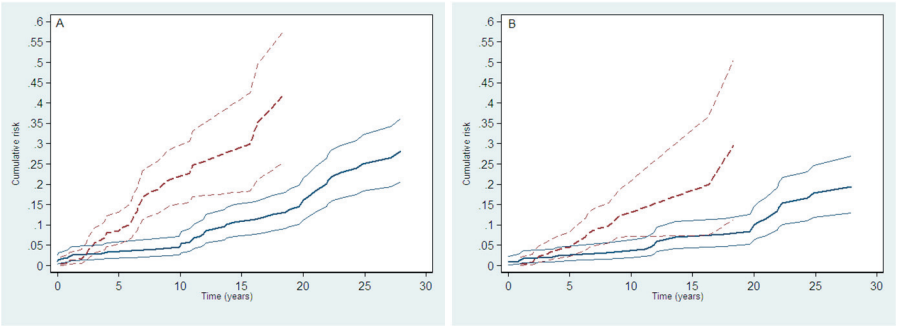


Figure 10. Cumulative risk of colorectal neoplasia with 95% confidence intervals before and after liver transplantation in PSC patients with IBD. A) Risk of low grade dysplasia, recurrent low-grade dysplasia, DALM, high grade dysplasia and carcinoma combined before (solid line) after (dashed line) liver transplantation. B) Risk of DALM, high-grade dysplasia and carcinoma combined. Before (solid line) and after (dashed line) liver transplantation.

Using a normal Cox regression analysis the corresponding hazard rates were 4.2 (95% CI 2.5-6.98), $p \leq 0.001$ for any type of neoplasia and 3.1 (95% CI 1.56-6.03) $p = 0.001$ for DALM, HGD and cancer combined. Patient age did not significantly contribute to the combined differences. To investigate if a longer IBD duration contributed to the increased estimated risk, we categorized the patients into two groups according to their IBD duration (less or more than 10 years). The risk was slightly higher in the group with more long-standing IBD but the risk did not reach statistical significance, HR 1.34, 95% CI 0.93-1.93, $p = 0.115$. A plot of the cumulative hazard as a function of duration of IBD, showed a higher hazard for the entire IBD course than for that before OLT.

4.3.5 Risk factors for colorectal neoplasia after OLT in PSC patients with IBD

A total of 189 patients still at risk of colorectal neoplasia after OLT had a post transplant follow-up time of at least 2 years. Colorectal neoplasia was diagnosed in 33 of these patients during follow-up. A univariate analysis identified aminosalicylates, UDCA and tacrolimus as significant risk factors for neoplasia, and they all remained significant in the multivariate model. The duration of use of aminosalicylates, UDCA and tacrolimus was median 24 (12-48), 24 (12-48) and 24 (12-24) months respectively.

Duration of IBD before transplantation was associated with risk of neoplasia but with borderline significance in the univariate analysis (p = 0.064), and lost its significance in the multivariate model. IBD activity did not contribute to an increased risk of colorectal neoplasia.

4.4 HIGH DOSE URSODEOXYCHOLIC ACID IN PRIMARY SCLEROSING CHOLANGITIS DOES NOT PREVENT COLORECTAL NEOPLASIA (PAPER IV)

4.4.1 Patient characteristics

Ninety-eight patients were included in the study; median age at inclusion in the UDCA trial was 39 years among UDCA treated patients and 43 in the placebo group. The two groups were similar in terms of sex, age at onset of IBD, type of IBD and colonic surveillance. The intensity of colonoscopic surveillance during the five-year trial was similar in the two groups with an average of 2.3 colonoscopies (range 1-7) in UDCA-treated patients and 1.8 (range 1-8) in the placebo group. The frequency and indication for colectomy were similar in the two groups. Information on family history for colorectal cancer was available in 73% (72/98), and similar in treated and untreated patients.

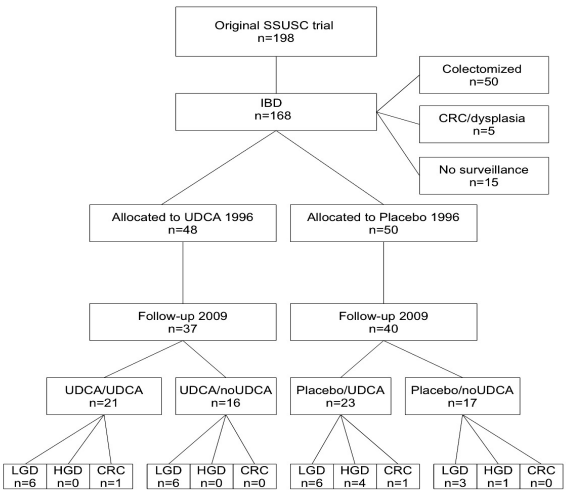


Figure 11. Selection and outcome of patients in the study. SSUSC = Scandinavian Study of UDCA in Primary Sclerosing Cholangitis

4.4.2 Colorectal neoplasia at the closure of the trial

Five patients died during the first five years of the study, two in the UDCA group (CCA in both patients) and three in the placebo group (CCA in two and one due to liver failure). At the closure of the randomized trial 13% (n=6) of the UDCA-treated patients and 16% (n=8) of the placebo patients had reached the combined endpoint of dysplasia or cancer. In each group there were five patients with LGD and one patient with CRC. However, HGD was only found in two of the patients in the placebo group. Comparison of the cancer- and dysplasia-free survival using the Kaplan-Meier method revealed no difference between the UDCA-treated and placebo patients (p=0.46, log rank test).

4.4.3 Colorectal neoplasia at the extended follow up in 2009

After closure of the original trial, 21 of the 37 UDCA treated patients available for follow-up continued the treatment. In the placebo group 23 of 40 patients had received treatment after 2001. The decision to continue or end treatment was made by the local investigators and the reasons are therefore not known. In the UDCA group 19 of the 21 patients were treated with high-dose UDCA; in two patients the dose was lower than 17 mg/kg/day. In the placebo group 22 of 23 patients were put on high-dose UDCA, and in one patient the dose was unknown. In total 28 patients (29%) developed colorectal dysplasia or cancer, 13 (27%) in the UDCA group and 15 (30%) in the placebo group. There was no detectable difference in dysplasia- and cancer-free survival when the groups were compared using the Kaplan-Meier method (p = 0.73 log-rank test).

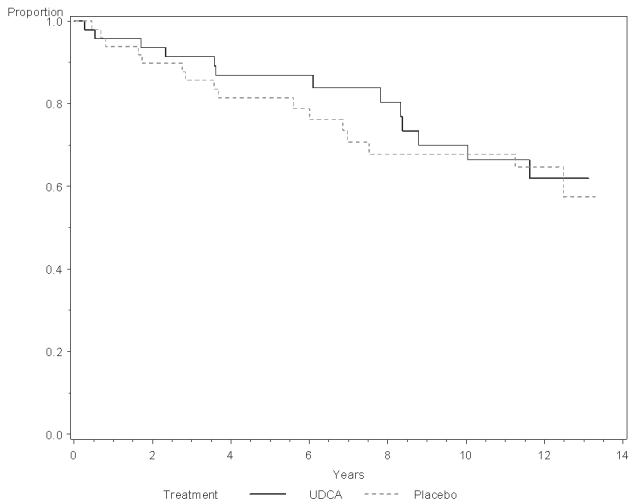


Figure 12. Dysplasia- and cancer-free survival after 12 years in 98 PSC patients randomized to treatment with UDCA vs. placebo for 5 years. *P* = 0.73, log rank

We performed an additional analysis where only HGD and cancer were considered endpoints. In this analysis we found a lower prevalence of dysplasia and cancer in patients initially assigned to UDCA but the difference did not reach statistical

significance ($P = 0.07$ log rank). We also did a comparative analysis of the patients who had never been treated with UDCA (n = 17) versus those who were treated until the end of follow-up or until reaching an endpoint (n = 21). No difference was seen between these two groups regarding cancer- and dysplasia-free survival ($P = 0.49$, log rank).

4.4.4 Covariates associated to cancer or dysplasia

A univariate analysis showed that the only covariate associated to cancer or dysplasia was the number of colonoscopies performed ($P < 0.0001$). No differences were detected regarding age at onset of IBD, IBD duration, and treatment with 5-ASA, UDCA or family history of CRC.

4.5 A REDUCTION IN ALKALINE PHOSPHATASE IN PRIMARY SCLEROSING CHOLANGITIS IS ASSOCIATED WITH A BETTER PROGNOSIS REGARDLESS OF TREATMENT WITH URSODEOXYCHOLIC ACID (PAPER V)

4.5.1 Patient characteristics

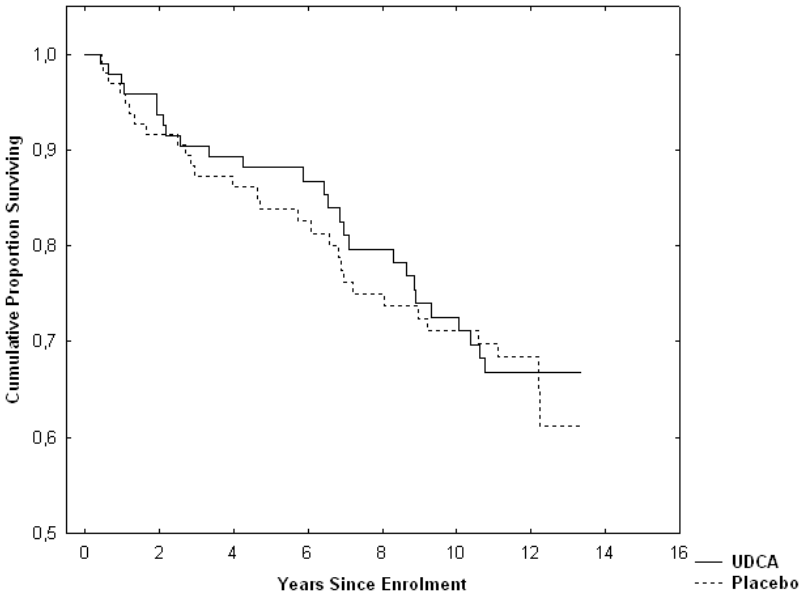
Overall, 198 patients were included in the study; 97 were treated with UDCA and 101 with placebo. Treated and untreated patients were similar regarding sex, presence of IBD, age at enrolment, duration of PSC at enrolment, follow-up years and levels of ALP at enrolment.

4.5.2 Outcome at follow-up in 2009/2010

Fifty-five patients reached an endpoint at the extended follow-up, 26 in the UDCA group and 29 in the placebo group. Twenty-nine patients were liver transplanted, 14 patients died and 12 were diagnosed with cholangiocarcinoma (all died after the diagnosis). The frequencies of the endpoints were similar in patients originally assigned to UDCA and placebo.

4.5.3 UDCA vs. placebo

No difference in endpoint-free survival was detected between UDCA treated and untreated patients (Fig. 12).



UDCA	**97**	**84**	**78**	**56**	**51**	**20**
Placebo	**101**	**84**	**72**	**59**	**56**	**19**

Figure 13. Kaplan-Meier survival curve of 198 patients enroled in the five-year Scandinavian UDCA trial in 1996 with extended follow-up in 2009/2010 ($P = 0.77$)

There was no difference in UDCA treatment after the end of the original trial; in total 40% of the patients were treated with open label UDCA after the end of the randomized period, 55% of the patients originally assigned to UDCA and 48% of the patients in the placebo group.

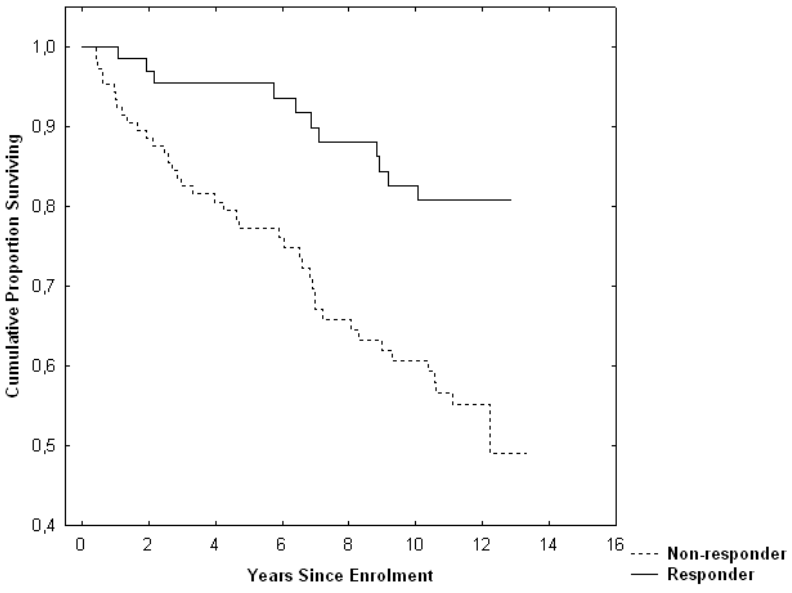
4.5.4 Survival in biochemical responders vs. non-responders

We categorized the UDCA treated patients into biochemical responders (n=43) and non-responders (n=51). All patients could be categorized except three patients for whom both the screening value and the one-year value were missing; these patients were excluded from further analysis. Using the Kaplan-Meier method we found that patients with an ALP reduction after treatment with UDCA had a significantly better survival rate than those who did not respond ($P = 0.033$, log rank).

The finding of improved survival in responders to UDCA intrigued us to further investigate if this finding was a consequence of the UDCA treatment or if ALP reduction per se was important for survival. Therefore we decided to categorize all patients into responders (n=79) and non-responders (n=116), regardless of UDCA treatment.

4.5.5 ALP responders vs. non-responders

Comparison between the groups revealed a highly significant difference in the endpoint-free survival between patients that reduced their ALP compared with those who did not ($P = 0.0001$, log rank).



Responders	**79**	**72**	**69**	**56**	**53**	**17**
Non resp.	**116**	**93**	**78**	**56**	**52**	**21**

Figure 14: Kaplan-Meier analysis of endpoint-free survival in biochemical responders vs. non-responders, regardless of treatment with UDCA ($P = 0.0001$, log rank)

The highest endpoint-free survival rate was found in the patients in the original placebo group who had a reduction of ALP, with a cumulative proportion surviving of 0.9 after 12 years as compared to the poorest survival in UDCA-non-responders of 0.47 ($P = 0.001$).
Some patients (22%, n=43) had consistently normal ALP levels at screening and after one year in the trial, and when these were excluded, a significant difference in long-term survival remained between patients that actually reduced their ALP levels and those who did not ($P = 0.03$, log rank).

5 GENERAL DISCUSSION

The general objective of this thesis was to investigate inflammatory bowel disease in patients with primary sclerosing cholangitis. We aimed to explore IBD in PSC patients before and after liver transplantation and to investigate what the characteristics of Crohn's disease in PSC are.
IBD in PSC is known to be associated to a high risk of colorectal neoplasia, but the published results regarding the risk after transplantation were contradictory. Moreover, the risk of CRN in PSC patients with Crohn's disease had never previously been studied. Treatment of PSC with ursodeoxycholic acid has been controversial for more than a decade, and the drug is widely used despite a total lack of evidence for beneficial effects regarding disease progression. UDCA has also been believed to work as a chemopreventive agent on colorectal neoplasia. The Scandinavian UDCA trial is to date the largest randomized controlled trial on UDCA in PSC. We hoped that a follow-up of the complete cohort could provide evidence for or against UDCA use in PSC, and shed some light on both long-term survival and the risk of colorectal neoplasia.

5.1 THE PHENOTYPE OF IBD IN PSC - CROHN'S DISEASE AND INFLAMMATORY ACTIVITY

In previous studies on PSC-IBD some of the patients were reported to suffer from Crohn's disease. Most of them have been described to have colonic involvement without features typical of Crohn's disease such as fistulas, deep ulcers or granulomas [55]. Our study was the first in which a large cohort (n=28) of PSC-CD was investigated to establish the phenotype of PSC-CD. In our study we found that classical features of Crohn's, such as obstructing disease and perianal fistulas, were rare and few patients showed definite histological signs of Crohn's (Paper I). Our patients also demonstrated less need for surgical intervention than patients without PSC.

Recently another report concerning a PSC-CD cohort (n=32) from Great Britain was published by Halliday et al [108]. They found, in line with our results, that patients with CD in PSC had less ileal involvement than patients with CD alone and that fewer PSC-CD patients were smokers. They could not confirm our finding of different need for surgical interventions in PSC and non-PSC-CD patients. In contrast to our findings the British study could not detect any difference between obstructing or perianal disease in CD patients with and without PSC. Why our cohorts were different with regard to perianal or obstructing CD is an open question but one might speculate that differences in patient selection, diagnostic methods, ethnicity and medication may have influenced the outcome.

To minimize confounders we chose to use matching in our study. Another study design might have included the use of randomization, restriction, stratification or correcting for confounders in a regression analysis. However, in view of the small size of our sample, the most efficient way to study possible differences between these two groups was to use matched cohorts. We believed that matching and scrutinization of individual medical records would make the results more reliable than if we used register data.

Diagnosis of colonic Crohn's disease is known to be difficult in patients with primary colonic involvement. The diagnosis of CD in our study was based upon the Lennard Jones diagnostic criteria published in 1989. They are considered to be incomplete, but are still the ones recommended in the ECCO guidelines for diagnosis of Crohn's disease [109]. There is a small possibility that our patients were actually misclassified patients with UC. However, the fact that all patients were diagnosed by gastroenterologists, and the thorough scrutinization of medical records, makes this unlikely.

The low prevalence of CD in PSC and the small number of patients with typical diagnostic features such as fisulas and granuloma raises the question if CD actually exists in PSC. Our results, together with the recently published paper by Halliday et al, demonstrate that CD with primarily colonic involvement is present in PSC. However, diagnosing CD in patients with PSC is difficult and better diagnostic means would be of value. The differentiation between CD and UC is important since it may influence the choice of medical treatment but has even a greater importance for selecting the most suitable type of surgery for IBD.

5.1.1 Inflammatory IBD activity in PSC

PSC per se appears to have a beneficial effect in terms of IBD activity. Patients with PSC typically have a mild symptomatic or even asymptomatic IBD, with less need for medication and surgery. Patients usually have long remissions and a quiescent course of their colonic disease [55, 58, 110].

Smoking is a factor that is well known to influence the activity of IBD. Smoking is associated with development of Crohn's disease, and is correlated to greater need for immunosuppressive treatment, including steroids, and surgery [111]. Conversely, smoking protects against development of ulcerative colitis and after disease onset eases the course of UC and decreases the risk of colectomy. In our study on CD in PSC (Paper I) we found that fewer patients with CD (14%) and PSC were smokers compared with patients with CD (46%) alone, suggesting that smoking may play a protective role in the development of PSC in patients with Crohn's disease. The low smoking frequency in the PSC-CD patients is consistent with studies on smoking behaviour in PSC in general [17, 18].

Studies on the inflammatory activity after liver transplantation have shown contradictory results [112, 113].

Our study is the largest cohort on PSC-IBD activity after OLT. We found that the activity of IBD in PSC patients increased after liver transplantation in terms of colonic inflammation, number of relapses and overall IBD activity (Paper II). We also saw a trend toward an increased risk of colectomy due to high disease activity after OLT. Only 11 (5%) of the patients were smokers, of whom 10 continued to smoke after OLT, which makes it unlikely that smoking influenced the IBD activity.

Earlier studies have reported that the majority of PSC patients had reduced IBD activity post OLT [113-115]. These studies were small (including in total less than 100 patients), used different statistical methods and were performed in the time period when use of CsA and azathioprine was more common. We found that a combination treatment with CsA and azathioprine lowered IBD activity or left it unchanged. Our finding is in accordance with another study by Haagsma and co-workers [112] of 48 transplanted PSC patients, where azathioprine appeared to have a protective effect against inflammation post OLT.

Both CsA and azathioprine are established IBD treatments. In our study cyclosporine A and azathioprine showed a protective effect against IBD flares, whereas the disease activity was shown to increase in patients in patients using MMF and tacrolimus. Therefore it is apparently not the immunosuppression per se but rather the choice of drugs that seems important. Like CsA and azathioprine, tacrolimus and MMF are also established treatments in IBD and our finding is hard to explain, but tacrolimus has previously been suggested to increase disease activity post OLT in IBD [112, 116]. We cannot ascertain whether the association with increased IBD activity after transplantation is due to tacrolimus or MMF or a synergistic effect of this combination. The observation that PSC –IBD responds differently to immunosuppression than patients without PSC further strengthen that IBD in PSC is a unique phenotype.

The Nordic transplanted PSC cohort studied in paper II and III is the largest series of patients with IBD and PSC (n = 439) undergoing liver transplantation so far reported. One strength of the study is that disease activity was assessed with different modalities. Also the competing risk regression analysis is perhaps the most proper way of estimating risk of colectomy in these patients; an overestimation of the risk is less likely with this than with other models (Kaplan-Meier and Cox-regression) that do not take informative censoring into account [107]. One of the limitations of the study is the retrospective design and the multiplicity of endoscopic examiners with the possibility of a heterogeneous estimation of disease activity. The possibility that some patients with increased IBD activity were actually misclassified patients with CMV or MMF colitis cannot completely be excluded; however, careful assessment of patient's records minimizes the risk of misclassification.

A shift from tacrolimus and MMF to CsA and azathioprine in liver transplanted PSC-IBD patients seems beneficial, but future studies will be needed to confirm this strategy. Differences in other important outcomes such as graft and patient survival after OLT, and adverse effects of CsA compared to tacrolimus need to be considered.

5.2 THE RISK OF COLORECTAL NEOPLASIA IN PSC-IBD

5.2.1 Colorectal dysplasia and cancer in Crohn's disease

In our study on CD in PSC using a study design similar to that of Broomé in 1995, we presented for the first time data that support an increased risk of colorectal neoplasia also in patients with PSC and Crohn's disease.

Broomé was the first scientist to suggest that patients with UC and PSC are at increased risk of developing CRN [66]. A few years later Broomé and co-authors published what has been referred to as the landmark study on risk of CRN in PSC, showing a cumulative risk of developing CRN in PSC-UC patients after 10, 20 and 25 years of disease duration of 9%, 31% and 50% respectively [64]. Since then primary sclerosing cholangitis is considered an independent risk factor for colorectal neoplasia in ulcerative colitis [65]. It is known that patients with Crohn's disease with more UC-like features and primarily colonic involvement are at greater risk of CRN than other CD patients [117]. This is in line with the clinical phenotype of CD that we found in our study.
Braden et al, on the contrary, stated that patients with Crohn's disease and PSC do not have an increased risk of colorectal malignancies [118]. One limitation is that unlike ours, the Braden study excluded patients with a short duration of PSC (less than 3 years) and IBD (less than 5 years) and some cases of colorectal neoplasia may therefore have been undetected. There have been reports of CRC developing soon after diagnosis in PSC [119].
Thus, patients with PSC and CD appear to be at higher risk of CRN than patients with CD alone, and patients with PSC-IBD should be considered risk patients for colorectal cancer and included in surveillance programs regardless of phenotype.

5.2.2 The risk of colorectal neoplasia after liver transplantation

One of the theoretical mechanisms behind CRN in PSC is cholestasis. Liver transplantation could therefore in theory protect against CRN since the cholestasis is corrected by replacement of the liver.
In our multicentre study of liver transplanted PSC patients with IBD we found that patients with PSC and IBD have an increased risk of colorectal neoplasia after liver transplantation, a risk that is even higher than before OLT (Paper III).

Previous studies showing an increased risk of post-OLT colorectal malignancies are not completely comparable to ours in terms of study design and statistical methods. Vera et al reported an incidence of colorectal cancer of 5.3% vs. 0.6% when comparing 152 liver transplanted PSC patients with 1184 non-PSC cases [120].
Dvorchik et al reported a lower incidence of colorectal cancer after OLT, but included only colorectal cancer in their analysis [100].

We defined colorectal neoplasia as epithelial dysplasia or cancer, assuming that dysplasia is a premaligant stage that leads to CRC if left untreated.

One problem with studying colorectal cancer risk in this population is the high number of competing events such as death or colectomy for other causes. Previous studies both on the risk of CRN in general in PSC and after OLT may have overestimated the risk. Improvements in IBD treatment and surveillance during the past years may also have influenced the current risk of CRC in PSC, which in general appears to be decreasing [121].
Our hypothesis was that the type of immunosuppression given after OLT could influence the risk of CRN in transplanted IBD patients. A univariate analysis could identify aminosalicylates, UDCA and tacrolimus as significant risk factors for neoplasia, whereas CsA had a protective effect against CRN. However only aminosalicylates and UDCA remained significant in the multivariate model. Since the transplanted patients always use either tacrolimus or CsA is it impossible to draw a conclusion whether it is tacrolimus that promotes or CsA that protects against colorectal neoplasia.
It seems clear however that the risk of CRN remains and appears increased after a liver transplantation and that these patients should be included in surveillance programs even after OLT. The immunosuppressive treatment, the IBD treatment and the use of UDCA for PSC patients after OLT demands further investigation.

5.2.3 Surveillance

The objective of coloscopic surveillance is to identify early neoplastic changes such as LGD and HGD of the colonic mucosa by yearly colonoscopies with biopsies and offer the patient a prophylactic colectomy before cancer develops. The role of colonoscopic surveillance for CRC is controversial in IBD without liver disease, and the cost-effectiveness of this strategy has been questioned.

The risk of progression from LGD to HGD in IBD patients is under debate. In PSC, the risk of progression from LGD to HGD is increased [122]. This supports the recommendation of colectomy in PSC patients with colonic LGD and certainly those with HGD.

The main cause of death in PSC is liver failure, but life expectancy is also seriously threatened by the unpredictable occurrence of CCA and the risk of colorectal carcinoma. One study has showed that the lifetime risk for CRC is in fact higher than for CCA in patients with PSC and IBD [123]. In view of the high risk of cancer, the rationale for colonic surveillance in PSC-IBD seems correct, and according to our results, surveillance should be practiced also in liver transplanted patients and in patients with Crohn's disease.

The efficacy of surveillance for detection of early malignancies could also be questioned from the standpoint of cost and adherence. A population-based analysis of the cost and practice of colonic surveillance of patients with PSC in Alberta, Canada revealed that only 1/3 of the colonoscopies expected were actually performed, but despite suboptimal surveillance, the incidence of colorectal neoplasia was high.

The study also found that the cost of finding one additional case of dysplasia was substantial [124].

In the study of CD in PSC (Paper I) we found that all patients had undergone at least two colonoscopies with a mean of five during the observation period (median 12 years). In the control group, however, six patients had to be excluded because they had not had at least two colonoscopies. This points at an important problem in clinical practise: that doctors' and patients' adherence to surveillance programs is not always easy to maintain. Surveillance of some patients may therefore be sporadic also in Scandinavia, and studies on the cost-effectiveness of colonic surveillance in PSC are lacking.

5.2.4 The mechanism behind the increased risk of colorectal neoplasia in PSC

Unlike sporadic CRC, cancer in IBD arises from chronic inflammation and is probably caused by a different mechanism. It has been shown that IBD-related CRN follows a different sequence of genetic alterations than sporadic CRN [125]. The mechanism behind CRN in PSC-IBD lies beyond the scope of this thesis and is largely unknown; however, some theories are worth mentioning. The fact that most CRN arises in the right side of the colon (Paper I, III) may suggest a different pathogenesis than cancers in IBD without PSC. Most investigators have focused on the premise that the composition of bile acids plays a major role in the pathogenesis. However, patients with PSC without IBD do not have an increased risk of CRN, suggesting that the composition of bile in combination with an inflamed colon is part of the mechanism. Genetic factors probably also play a role; and at least two larger GWAS studies are ongoing and may shed some light on the pathogenesis.
Evidence has also been presented to support a role of the intestinal flora in the pathogenesis of colitis-associated cancer [126]. There is also a close relationship between gut flora and bile salts; perhaps changes in bile acid composition may favour more carcinogenic bacterial strains in the colon of patients with PSC. Future studies are needed to explore the pathogenesis behind colorectal neoplasia in PSC.

5.2.5 UDCA as a chemopreventive agent for CRN

The main finding of Paper IV was that 5 years of treatment with UDCA at a dose of 17-23 mg/kg/day did not have an impact on the long-term risk of CRN in PSC patients.

Chemopreventive treatment in PSC is an attractive option, due to the high risk of cancer in these patients in general, but studies designed for chemopreventive purposes are few. Large prospective studies are very difficult to perform in PSC because of the rareness of disease and the large proportion of patients that will die of liver-related causes.

The effect of UDCA and the development of colorectal neoplasia appear to be dose-dependent. In a study by Alberts et al a low dose of UDCA (8-10 mg/day) prevented recurrence of adenomas in patients with non-PSC CRN [127].

Also the study by Pardi et al using a lower dose than the dose in Paper IV showed a preventive effect of UDCA [89]. Our study using a moderate dose of 17-23 mg showed no effect of UDCA and the very high dose used in the trial by Eaton et al of 28-30 mg/kg was even associated with an increased risk of neoplasia [90, 92].

The ideal way to study the chemopreventive effect of UDCA in PSC would be to design a large RCT intended to evaluate not only survival but also the risk of CRN, and in which colonoscopies would be done in a standardized way on all patients. It is not likely that such a study will ever be performed and the retrospective analysis of the Scandinavian trial is therefore of great value. Most patients in Paper IV had regular colonoscopies, but due to the retrospective study design, we may have missed some cases of neoplasia. A colonoscopy is not the perfect way to find neoplasia, especially not the flat type of lesions common in PSC, but it remains the best option today [128]. Hopefully new methods, such as laser-based endomicroscopy, the use of immune surveillance or perhaps even faecal markers for dysplasia will provide a more convenient and safe way to diagnose neoplasia early in PSC and in other patient groups with high risk of colorectal cancer.

Another issue with paper IV that must be addressed is the risk of confounding by indication, a possible bias. After the randomized period, many patients were treated with UDCA regardless of which group they were originally assigned to. Outside a randomized trial it is challenging to do a valid comparison between drug takers and non-takers. The problem arises from the fact that those who take a drug generally differ from those who do not, so called confounding by indication. We cannot exclude that patients who were treated with UDCA after the trial differed significantly in terms of both disease severity and risk of colorectal neoplasia compared to those who were not treated. The strength of Paper IV lies in the fact that it is based upon the largest-ever trial on UDCA in the dose normally given in the clinical setting, and the fact that the first five years were done as an RCT.
Based on our results the use of UDCA at a dose of 17-23 mg/kg cannot be encouraged as a chemopreventive strategy in PSC-related IBD. Evaluating UDCA administration at lower doses may still be interesting, but in view of the risk linked to higher doses, a recommendation of low-dose UDCA to PSC patients seems unlikely in the future.

5.3 UDCA AS A TREATMENT IN PSC

5.3.1 UDCA, biochemical response and long-term survival in PSC

In the follow-up study of the Scandinavian UDCA trial we found no benefit of UDCA treatment for patients with PSC regarding long-term transplant-free survival (Paper V). Together with other studies using different doses [83, 86] there is today no evidence that treatment with UDCA slows the progression of PSC. This has already had an impact on recently published guidelines that discourage the use of UDCA in PSC [14].

Moreover, as a secondary finding, we saw that patients who reduced their levels of ALP by ≥40% had significantly better long-term survival than patients who did not: the reduction of ALP was associated with a better prognosis regardless of treatment.

Several studies have shown that fluctuations in ALP occur naturally in PSC and appear to occur independently of the presence of dominant strictures on cholangiograms [129]. In 2011, Stanich et al published a study including 87 newly diagnosed patients with PSC and reported that ALP normalization was associated with a better prognosis in PSC, independently of UDCA treatment or bile dilatations [130]. Our study includes patients with different disease durations but a median of 6 years at the time of entry into the trial, so it appears as if a significant reduction in ALP, independent of disease duration, is associated to a better outcome. The strengths of our study are the long follow-up time, the large cohort and that UDCA was given in a randomized controlled trial during the first five years.

ALP measurement is a routine laboratory test commonly used to diagnose patients with suspect PSC. We found that an apparently spontaneous reduction of ALP was associated with a significantly better outcome in our patients. ALP is not included as a prognostic parameter in the two most commonly used prognostic models of PSC: the Mayo natural history model and the time-dependent Cox regression model. The prognostic models are difficult to apply in the clinical setting and are not recommended for predicting the prognosis of an individual patient. Future studies will have to evaluate the use of ALP in the prognosis and follow-up of our patients and ALP should be considered for use as a prognostic marker when evaluating new drugs for PSC.

5.3.2 Summary and perspectives for the future

Our studies show that patients with IBD and PSC have a high risk of developing CRN regardless of IBD phenotype, and that the risk of CRN and IBD activity increases after OLT and appears correlated to the type of immunosupression given. The evidence that UDCA improves survival in PSC or that it should be used as a chemopreventive agent in PSC-IBD is weak. Future studies will focus on optimization of diagnosis and surveillance of PSC-IBD, look into the pathogenesis of CRN in PSC in order to evaluate new drugs, and attempt to optimize the immunosupression given after a liver transplantation in PSC. Alkaline phosphatase is a marker for disease progression in PSC and should be used in future clinical trials.

6 CONCLUSIONS

Crohn's disease in patients with primary sclerosing cholangitis is characterized by colonic involvement, and a lack of both obstructive disease and perianal fistulas. (Paper I)

Primary sclerosing cholangitis is a risk factor for development of colorectal neoplasia in Crohn's disease. (Paper I)

The activity of inflammatory bowel disease increases after a liver transplantation in PSC and appears influenced by the immunosuppression given. (Paper II)

The risk of colorectal neoplasia in patients with PSC and IBD increases after a liver transplantation. (Paper III)

Treatment with ursodeoxycholic acid in the dose of 17-23 mg/kg does not prevent colorectal neoplasia in PSC-IBD. (Paper IV)

Treatment with ursodeoxycholic acid does not improve the long-term survival in PSC. (Paper V)

A reduction in alkaline phosphatase is associated with a better long-term prognosis in PSC. (Paper V)

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Primär skleroserande cholangit (PSC) är en ovanlig leversjukdom som kännetecknas av inflammation och ärrbildning i små och stora gallgångar. Sjukdomen har en förekomst på omkring 10 på 100 000 i Norden och två tredjedelar av patienterna är män. Sjukdomen debuterar oftast hos unga vuxna, men förekommer även hos barn. Medianåldern vid insjuknande är 40 år.
Orsaken till PSC är fortfarande okänd, även om mycket talar för att det är en sjukdom med immunologisk/genetisk bakgrund. Diagnosen ställs genom en kombination av blodprover samt typiska förändringar på röntgenundersökning (s.k. kolangiografi) samt i prover från levervävnad.
Många patienter är symtomfria men typiska symtom på PSC är smärta under höger revbensbåge, gulsot, feber, klåda och viktnedgång. Sjukdomen förvärras över tid och leder oftast till leversvikt som kräver levertransplantation eller gallgångscancer. Tiden till behov av levertransplantation efter diagnos varierar, men är i medeltal 12 år Medicinsk behandling som bromsar utvecklingen till skrumplever saknas helt. Den enda potentiellt botande behandlingen för en patient med PSC och sviktande leverfunktion är en levertransplantation.
Levertransplantationer har i Sverige utförts sedan 1984 då den första transplantationen ägde rum på Huddinge Sjukhus, och PSC är i nuläget den vanligaste indikationen för levertransplantation i de Nordiska länderna.
De flesta patienter med PSC har samtidigt en inflammatorisk tarmsjukdom, där ulcerös kolit är vanligast. I Sverige har ca 80 % av PSC patienterna samtidig inflammatorisk tarmsjukdom, varav 80 % har ulcerös kolit. Ett antal patienter diagnosticeras dock med en annan typ av, Crohn´s sjukdom. En klinisk iakttagelse är att Crohn´s sjukdom vid samtidig PSC inte uppvisar typiska kännetecken som anala fistlar eller uttalad tunntarmssjukdom. Inflammatorisk tarmsjukdom vid PSC kännetecknas av få eller inga tarmsymtom, en låggradig men total inflammation i tjocktarmen och av ökad risk för tjocktarmscancer. Patienter med ulcerös kolit har en ökad risk att utveckla cancer i tarmen, förekomst av PSC ökar denna risk ytterligare. Risken för tjocktarmscancer hos patienter med PSC och Crohn´s sjukdom har däremot inte studerats tidigare.

Efter en levetransplantation behandlas patienter med starka immunhämmande läkemedel för att förhindra avstötning av den nya levern. En del PSC patienter som levertransplanterats får trots en stark immunhämmande behandling ökad inflammation i tarmen efter transplantationen. Orsaken är oklar och i små studier har behandling med det immunhämmande läkemedlet takrolimus efter transplantation föreslagits vara en möjlig orsak. Risken för tjocktarmscancer kvarstår efter levertransplantation men det är oklart om risken för att utveckla tjocktarmscancer är ännu högre hos transplanterade PSC patienter.

Ursodeoxycholsyra (UDCA) är en vattenlöslig gallsyra som naturligt finns i låga nivåer i galla och har visat sig ha egenskaper som skyddar mot cancer. Att ge extra, syntetiskt framställd, UDCA har studerats vid PSC och har visat sig förbättra leverprover men någon ökad överlevnad eller säker effekt på sjukdomens förlopp har inte kunnat visas.

Ett litet antal studier har undersökt sambandet mellan intag av UDCA och risken för tjocktarmscancer, men resultaten är motstridiga och långtidsdata saknas. I klinisk praxis används preparatet trots detta brett.

Att genomföra bra studier hos patienter med PSC är svårt. Framförallt är det en ovanlig sjukdom vilket gör det svårt att samla in tillräckligt stora patient material för att kunna påvisa en statistiskt signifikant skillnad. Sjukdomen är dessutom oförutsägbar och markörer som kan förutsäga prognosen saknas.

Det övergripande målet för denna avhandling var att studera karakteristika av inflammatorisk tarmsjukdom och risk för kolorektal cancer före och efter levertransplantation hos patienter med PSC. Vi ville också studera effekten av UDCA behandling för utveckling av kolorektal cancer och progress av leversjukdomen.

I den första studien har vi kartlagt Crohn´s sjukdom hos patienter med PSC och beskrivit skillnader och likheter med patienter med Crohn´s sjukdom utan PSC. Vi fann att typiska tecken på CD ofta saknades hos patienter med PSC. Andelen rökare, förekomsten av anala fistlar, inflammation som orsakar svår ärrbildning och behovet av kirurgi var signifikant mycket mindre hos patienter med samtidig PSC. Däremot fann vi att risken för att utveckla tjocktarmscancer var större hos patienter med PSC jämfört med de leverfriska patienterna.

Studie II och III i denna avhandling inkluderar samtliga nordiska patienter med PSC som levertransplanterats. Totalt 439 patienter ingick i studien och följdes från diagnos till död eller tills studien avslutades. Vi fann att den inflammatoriska aktiviteten i tarmen ökade efter transplantationen och att det fanns en koppling mellan aktivitet i tarmen och vilken typ av medicinering som användes efter operationen. Vi kunde dessutom visa att risken för att utveckla tjocktarmscancer inte bara kvarstår efter en levertransplantation utan dessutom är dubbelt så hög jämfört med risken före kirurgi. Risken för tjocktarmscancer var också kopplad till vilken typ av immunhämmande medicinering som patienterna fått där risken för cancer var högre hos patienter som fått takrolimus och lägre hos patienter som behandlats med cyklosporin.

De avslutande två studierna i avhandlingen undersöker UDCAs skyddande effekt på tjocktarmscancer samt huruvida UDCA har en effekt på överlevnad hos patienter med PSC. Studierna är en långtidsuppföljning av 198 skandinaviska patienter som ingick i en tidigare randomiserad studie mellan UDCA och placebo. Våra långtidsresultat visade att risken för tjocktarmscancer inte påverkades av behandling med UDCA samt att överlevnaden inte var bättre hos patienter som hade fått behandling med UDCA. Ett oväntat fynd var att en minskning av nivåerna av blodprovet ALP var kopplat till en bättre överlevnad hos patienter med PSC. Detta fynd kan innebär att man på ett bättre sätt kan bestämma prognosen hos patienter med PSC och avgöra vilka patienter som behöver en tätare övervakning samt att provet kan användas som en markör för behandlingseffekt och sjukdomsprogress i framtida studier.

Sammanfattningsvis är PSC en riskfaktor för utveckling av kolorektal cancer vid Crohn's sjukdom. Sjukdomsaktiviteten av inflammatorisk tarmsjukdom ökar efter transplantation och påverkas av immunosuppressiv behandling. Risken för kolorektal cancer ökar efter levertransplantation hos PSC patienter med samtidig inflammatorisk tarmsjukdom. Behandling med UDCA påverkar inte långtidsöverlevnad och minskar inte heller risken för kolorektal cancer.

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