Kirsten Boonstra was born in Amsterdam on April 10, 1986. She graduated from Bredero Lyceum in 2004, after which she attended medical school at the University of Amsterdam.

Four years later, in 2008, she started a four-month scientific internship under the supervision of Dr. Cyriel Ponsioen and Prof. Ulrich Beuers. This turned out to be the beginning of a PhD project at the Department of Gastroenterology and Hepatology of the Academic Medical Center, Amsterdam. During the following six years she worked on several clinical and experimental studies, focusing on the epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis, as well as the immunology of inflammatory bowel disease in patients with primary sclerosing cholangitis.

In February 2013 Kirsten continued her medical training.
Primary sclerosing cholangitis and primary biliary cirrhosis
Epidemiology, risk factors, and outcome

Kirsten Boonstra
Primary sclerosing cholangitis and primary biliary cirrhosis
Epidemiology, risk factors, and outcome
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Introduction

Parts of this introduction have been previously published (in Dutch):
Nederlands Tijdschrift voor Geneeskunde – 2010;154:A1476
Chapter 1

Large population-based studies on incidence and prevalence, as well as on the natural history of the major chronic cholestatic liver diseases - primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) - are scarce, but very important for appropriate patient counselling and finding clues to etiology of the diseases. In 2006 we initiated a multicenter study in 44 hospitals in the Netherlands to obtain precise population-based prevalence and incidence figures; insight into disease course with regard to survival; the need for liver transplantation; and occurrence of malignancies, as well as risk factors thereof.

**PRIMARY SCLEROSING CHOLANGITIS**

PSC is a cholestatic liver disease of unknown etiology. Chronic inflammation of the intra- and extrahepatic bile ducts leads to bile duct destruction, cholestasis, liver fibrosis and progression to end-stage liver disease. From the moment patients are diagnosed with PSC, years of treatment with endoscopic dilatation, imaging, blood tests and annual colonoscopies will follow; and many patients ultimately need a liver transplant.

**Epidemiology**

PSC is more common in men than in women (2:1) and can manifest itself at any age, with a peak incidence around the age of 40. Up to 80% of PSC patients - depending on geographic location - have concomitant inflammatory bowel disease (IBD) with a distinct phenotype. Several studies have investigated the epidemiology of PSC using different case-finding and case-ascertainment strategies. The reported incidence and prevalence figures show notable variation, depending on the applied search strategy, the population under study, and the scrutiny of case-finding and ascertainment. The literature on incidence and prevalence rates around the globe is systematically reviewed in chapter 2 followed by an evaluation of the largest population-based PSC cohort to date in chapter 3.

**Diagnosis**

Roughly 50% of PSC patients present with symptoms such as pruritus, fatigue, pain in the right upper abdomen, or episodes of fever and cold chills. On physical examination, hepatomegaly and splenomegaly are the main findings, but these are found in a minority of patients. Blood analysis shows elevated alkaline phosphatase (AP) and γ-glutamyl transferase (γ-GT) levels, and an increased IgG concentration in more than half the patients. In 70% of patients serum bilirubin concentrations are not elevated at the time of diagnosis. Atypical perinuclear antineutrophil cytoplasmic antibodies (pANCA), anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies are linked to PSC, but are not disease-specific. The larger bile ducts, both intrahepatic and extrahepatic, are visualised using imaging techniques such as magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP). Until recently, ERCP was primarily used to diagnose PSC. A major disadvantage of ERCP is the risk of post-ERCP pancreatitis and/or cholangitis. Nowadays, the quality of non-invasive MRCP is usually sufficient to visualise the bile ducts without the disadvantages of an invasive procedure. Multifocal strictures and saccular dilatations of the intra- and/or extrahepatic bile ducts seen on cholangiography are hallmarks of PSC (fig. 1.1), although this so-called ‘beading’ can also be found in secondary sclerosing cholangitis due to cholelithiasis, biliary surgery, IgG4-associated cholangitis, and various other causes. Liver biopsy is of limited value. Often non-specific changes of the small bile ductules are found, and there is a high probability of sampling error. A liver biopsy may help if there is evidence of small-duct PSC or an overlap syndrome with autoimmune hepatitis. Small-duct PSC is characterised by cholestatic and histopathological features of PSC, but normal bile ducts on cholangiography. Other causes of bile duct strictures such as cholangiocarcinoma, recurrent bile duct stones or IgG4-associated cholangitis should be excluded before diagnosing PSC. The AASLD practice guideline on the diagnosis and management of PSC suggested measuring serum IgG4 in all patients with possible PSC, to exclude IAC. Chapter 6 addresses the diagnostic dilemma of elevated serum IgG4 in differentiating patients with a chronic cholangiopathy otherwise compatible with PSC or IAC.

**Figure 1.1** Multifocal strictures and saccular dilatations of the intra and extra hepatic bile ducts (beading)
Chapter 1

Pathogenesis

PSC is considered to be a complex genetic disease meaning that environmental factors trigger disease in a genetically predisposed individual. Several haplotypes of the HLA system have been associated with PSC, IBD and autoimmunity in general. Currently, 16 risk loci outside the HLA system have been identified in PSC. Some of these risk loci are associated with IBD, yet not all, suggesting a distinct genetic architecture. The strong association with IBD and the presence of gut-specific lymphocytes, chemokines, integrins, and addressins in liver biopsies and explanted livers of PSC patients has led to the hypothesis that PSC is an extra-intestinal manifestation of IBD. Colitis in PSC patients is reported to behave differently than other types of IBD in that it often runs a mild course, and the inflammation is predominantly located in the right colon. Chapter 4 reports how the phenotype of IBD in PSC patients was assessed using endoscopic and histopathologic criteria. IBD is considered to be the most important risk factor for PSC, yet little is known about environmental factors triggering the disease. In chapter 5, risk factors for developing PSC are assessed.

Disease course

Liver transplantation is the only available cure for PSC, although PSC can recur in the transplanted liver. Unfortunately, patients often die on the waiting list for liver transplantation, or they do not meet the criteria for receiving a donor liver. PSC patients are not only threatened by imminent liver failure, but also run an increased risk of developing colorectal cancer and cholangiocarcinoma. Survival, risk of malignancies and risk factors thereof, as well as the effectiveness of surveillance are described in chapter 3.

PRIMARY BILIARY CIRRHOSIS

PBC is a chronic cholestatic liver disease of the small and medium-sized intrahepatic bile ducts, affecting predominantly middle-aged or elderly women. PBC is considered to be an autoimmune disease due to the presence of antimitochondrial antibodies (AMA) directed against the E2 subunit of the pyruvate dehydrogenase complex, an enzyme complex found in mitochondria.

Epidemiology

PBC is more common in women than in men (male:female 1:9) with a peak incidence around the age of 60. Prevalence rates vary widely depending on location and search strategy. The incidence and prevalence rates around the world are reviewed in chapter 2. In chapter 7 we describe the population-based epidemiology of PBC in a large geographically defined area of the Netherlands.

Diagnosis

Similarly to PSC, about 50% of patients present with symptoms such as pruritus, fatigue or jaundice. The diagnosis of PBC is based on elevation of alkaline phosphatase (AP) of liver origin, and the presence of AMA in serum. Histological features of florid bile duct lesions support the diagnosis.

Pathogenesis

PBC is an autoimmune disease most likely triggered by environmental factors in genetically susceptible individuals. The striking female predominance in PBC and autoimmune diseases in general has led to the hypothesis that female-related factors may trigger disease. Intrinsic, environmental, and behavioural risk factors for PBC are assessed in chapter 7.

Disease course and treatment

In general, PBC patients respond very well to treatment with ursodeoxycholic acid (UDCA), a hydrophilic bile acid that has been shown to improve biochemical and histological markers, and subsequently to slow progression and improve survival. When medical therapy was not yet available for PBC, the estimated median survival from diagnosis until death was 8.4 years. Nowadays, the overall mortality is comparable to that in the general population, yet patients are at slightly increased risk of developing cancer, as discussed in chapter 8.

The unique setting in the Netherlands, with many people living in close proximity to high-quality care and excellent collaboration of gastroenterologists and hepatologists, allowed for inclusion of two large cohorts of PSC and PBC patients. This multicenter project enabled us to precisely study the epidemiology and disease course of PSC and PBC. The results of those studies are described in this thesis.
REFERENCES

Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review

Kirsten Boonstra, Ulrich Beuers, Cyriel Y. Ponsioen
INTRODUCTION

Primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) are enigmatic cholestatic liver diseases ultimately resulting in cirrhosis and liver failure. Both diseases are considered complex genetic diseases, but the etiopathogenesis is still unknown. PSC is more common in men than in women (2:1) and can occur at any age with a peak incidence around 40. PSC is strongly associated with inflammatory bowel diseases (IBD), most often ulcerative colitis, and patients have an increased risk for developing colorectal and hepatobiliary malignancies. The course of PSC is highly variable with reported median survival rates until liver transplantation or death from 12 to 18 years. PBC on the other hand predominantly affects middle aged or elderly women (1:9). Common symptoms associated with PSC and PBC are pruritus, fatigue and upper abdominal discomfort. However, more than 50% of patients are asymptomatic at time of diagnosis. Multifocal strictures and dilatations of the intra- and/or extra hepatic bile ducts seen on cholangiography are hallmarks for PSC, although these can also be found in secondary sclerosing cholangitis due to cholelithiasis, biliary surgery, IgG4 associated cholangitis or various other causes. Antimitochondrial antibodies directed against the E2 subunit of the pyruvate dehydrogenase complex are a sensitive serological hallmark for PBC. Ursodeoxycholic acid (UDCA) improves serum liver tests, histologic features and survival of PBC patients. In PSC, the beneficial role of UDCA in disease progression and survival is still unproven.

Several studies have investigated the epidemiology of both diseases, using different case-finding and case-ascertainment strategies. The reported incidence and prevalence figures show quite some variation, depending on the applied search strategy, the population under study, and the scrutiny of case-finding and ascertainment. We aimed to systematically review the literature on incidence and prevalence rates and temporal trends for PSC and PBC.

METHODS

Literature search

A systematic search of the medical literature was performed using the checklist proposed by the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group with assistance of a clinical librarian in peer-reviewed medical databases Medline and EMBASE (search last conducted January 10th, 2011). The following strategy was used to search Medline: ([“Cholangitis, Sclerosing”[Mesh]) OR (primary sclerosing cholangitis[tiab]) OR (“liver cirrhosis, biliary”[Mesh]) OR (primary biliary cirrhosis[tiab]) AND (“epidemiology”[subheading]) OR (epidemiol[tiab]) OR (“incidence”[Mesh]) OR (incidenc[tiab]) OR (prevalen[tiab]) OR
The search yielded 2286 abstracts of which 30 articles in English and one in Norwegian were eligible for inclusion. 2223 articles were excluded based on title and abstract. For the remaining 63 articles, reasons for exclusion are depicted in figure 2.1.

RESULTS

The search yielded 2286 abstracts of which 30 articles in English and one in Norwegian were eligible for inclusion. 2223 articles were excluded based on title and abstract. For the remaining 63 articles, reasons for exclusion are depicted in figure 2.1.

Study characteristics

Of included articles, nineteen reported incidence or prevalence rates in Europe,16-34 seven in North-America,35-41 three in Asia,42-44 and two in Australia.45,46 Studies varied in size from 10 to 770 patients in catchment areas from 100,312 to 19,230,000 inhabitants. Various sources had been used for case-finding purposes. In seventeen studies a search was performed in a medical record database using the International Classification of Diseases (ICD) or a similar diagnosis coding system. Other sources were laboratory databases, pathology databases, personal registry of physicians, radiological databases, hospital billing system and death certificates. Of the thirty-one included studies, thirteen (41.9%) used one source for case-finding, three studies (9.7%) used two sources, seven studies (22.6%) used three sources, five studies (16.1%) used four sources, two studies (6.5%) used five sources and one study (3.2%) combined six sources for case-finding. An overview is given in tables 2.1 and 2.2. A quality assessment of case-finding and case-ascertainment methods is presented in table...
### Table 2.1 Incidence and prevalence of primary sclerosing cholangitis

<table>
<thead>
<tr>
<th>Study</th>
<th>Period</th>
<th>No. of patients</th>
<th>Population</th>
<th>Case-finding</th>
<th>Case-ascertainment</th>
<th>Incidence per 100,000 (95% CI)</th>
<th>Prevalence per 100,000 (95% CI)</th>
<th>IBD</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escorsell [31] Spain</td>
<td>1984 - 1988</td>
<td>43</td>
<td>19,230,000</td>
<td>Personal registry gastroenterologists and hepatologists</td>
<td>I + II + III + IV</td>
<td>0.07</td>
<td>0.22</td>
<td>47%</td>
<td>60%</td>
</tr>
<tr>
<td>Berdal [24] Akershus, Norway</td>
<td>1985 - 1994</td>
<td>12</td>
<td>180,000</td>
<td>ICD-9</td>
<td></td>
<td>0.7</td>
<td>5.6</td>
<td>N/A</td>
<td>58%</td>
</tr>
<tr>
<td>Byron [35] Winnipeg, Canada</td>
<td>1987 - 1994</td>
<td>39</td>
<td>650,000</td>
<td>All clinical records referral center</td>
<td>II + III + VI or II + IV + VI</td>
<td>N/A</td>
<td>6.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Boberg [27] Oslo, Norway</td>
<td>1986 - 1995</td>
<td>17</td>
<td>130,000</td>
<td>Prospective registration</td>
<td>II + III + IV</td>
<td>1.3 (0.8-2.1)</td>
<td>8.5 (2.8-14.2)</td>
<td>71%</td>
<td>71%</td>
</tr>
<tr>
<td>Ang [44] Changi, Singapore</td>
<td>1989 - 1998</td>
<td>10</td>
<td>750,000</td>
<td>10 consecutive patients</td>
<td>II + IV</td>
<td>N/A</td>
<td>13</td>
<td>20%</td>
<td>90%</td>
</tr>
<tr>
<td>Bambara [38] Olmed County, United States</td>
<td>1976 - 2000</td>
<td>22</td>
<td>?</td>
<td>Medical records linkage system, pathology reports, laboratory reports, IBD research records</td>
<td>I + II + III + V or I + II + IV + V</td>
<td>0.9</td>
<td>13.6</td>
<td>73%</td>
<td>68%</td>
</tr>
<tr>
<td>Hursturt [37] Alaska, United States</td>
<td>1984 - 2000</td>
<td>0</td>
<td>100,312</td>
<td>All clinical records, ICD-9</td>
<td>I</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Card [32] United Kingdom</td>
<td>1987 - 2002</td>
<td>223</td>
<td>2,027,000</td>
<td>General Practice Research Database</td>
<td>N/A</td>
<td>0.41 (0.34-0.48)</td>
<td>3.85 (3.04-4.80)</td>
<td>48%</td>
<td>63.5%</td>
</tr>
</tbody>
</table>

Studies fulfilling all quality criteria regarding 1) definition of studied population; 2) case-finding method and 3) case-ascertainment criteria are highlighted in grey. a) case-ascertainment criteria: I: clinical features; II: serum AP↑≥ 6 months; III: ERCP or MRCP; IV: liver biopsy; V: no signs of secondary sclerosing cholangitis; VI: inflammatory bowel disease. ICD: International Classification of Diseases; IBD: inflammatory bowel disease; ERCP: endoscopic retrograde cholangiopancreatography; MRCP: magnetic resonance cholangiopancreatography.
### Table 2.2 Incidence and prevalence of primary biliary cirrhosis

<table>
<thead>
<tr>
<th>Study</th>
<th>Period</th>
<th>No. of Patients</th>
<th>Population</th>
<th>Case-finding</th>
<th>Case-ascertainment</th>
<th>Incidence per 100,000 (95% CI)</th>
<th>Prevalence per 100,000 (95% CI)</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamlyn [16] Newcastle upon Tyne, United Kingdom</td>
<td>1972 - 1977</td>
<td>117</td>
<td>2,080,000</td>
<td>personal registry physicians, positive AMA results, death certificates</td>
<td>I + IIb + III</td>
<td>0.9</td>
<td>N/A</td>
<td>7%</td>
</tr>
<tr>
<td>Triger [17] Sheffield, United Kingdom</td>
<td>1977 - 1979</td>
<td>34</td>
<td>520,000</td>
<td>personal registry physicians, positive AMA results</td>
<td>I + IIb + III</td>
<td>0.58</td>
<td>5.4</td>
<td>6%</td>
</tr>
<tr>
<td>Danielsson [18] Northern Sweden</td>
<td>1973 - 1982</td>
<td>11</td>
<td>570,000</td>
<td>personal registry physicians, hospital patient registry, positive AMA results</td>
<td>I + IIb or I + IIb + III</td>
<td>1.3</td>
<td>15.1</td>
<td>14%</td>
</tr>
<tr>
<td>Löfgren [20] Örebro, Sweden</td>
<td>1976 - 1983</td>
<td>18</td>
<td>164,063</td>
<td>positive AMA results</td>
<td>I + IIb + III</td>
<td>1.4</td>
<td>12.8</td>
<td>22%</td>
</tr>
<tr>
<td>Almdal [21] Denmark</td>
<td>1981 - 1985</td>
<td>233</td>
<td>5,100,000</td>
<td>hospital admission registry</td>
<td>I + IIb + III</td>
<td>0.9</td>
<td>N/A</td>
<td>24%</td>
</tr>
<tr>
<td>Myszor [22] Newcastle, United Kingdom</td>
<td>1965 - 1987</td>
<td>411</td>
<td>1,920,000</td>
<td>hospital admission registry, positive AMA results, personal registry physicians</td>
<td>I + IIb or I + IIb</td>
<td>1.98</td>
<td>15.35</td>
<td>10%</td>
</tr>
<tr>
<td>Witt – Sullivan [41] Ontario, Canada</td>
<td>1986 - 1988</td>
<td>225</td>
<td>?</td>
<td>personal registry physicians</td>
<td>I + IIb + III</td>
<td>0.9</td>
<td>N/A</td>
<td>24%</td>
</tr>
<tr>
<td>Watson [45] Victoria, Australia</td>
<td>1990 - 1991</td>
<td>84</td>
<td>4,390,000</td>
<td>personal registry physicians, hospital discharge registry, positive AMA results</td>
<td>I + IIb or I + IIb</td>
<td>N/A</td>
<td>191</td>
<td>8%</td>
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<table>
<thead>
<tr>
<th>Study</th>
<th>Period</th>
<th>No. of Patients</th>
<th>Population</th>
<th>Case-finding</th>
<th>Case-ascertainment</th>
<th>Incidence per 100,000 (95% CI)</th>
<th>Prevalence per 100,000 (95% CI)</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remmel [23] Estonia</td>
<td>1973 - 1992</td>
<td>69</td>
<td>1,526,177</td>
<td>personal registry physicians, positive AMA results</td>
<td>I + IIb + III</td>
<td>0.39</td>
<td>2.69</td>
<td>5%</td>
</tr>
<tr>
<td>Berdal [24] Akershus, Norway</td>
<td>1985 - 1994</td>
<td>21</td>
<td>180,000</td>
<td>ICD-9</td>
<td>I + IIb + III</td>
<td>1.2</td>
<td>12</td>
<td>0%</td>
</tr>
<tr>
<td>James [25] North-East England, United Kingdom</td>
<td>1987 - 1994</td>
<td>770</td>
<td>2,052,668</td>
<td>personal registry physicians, ICD-9, ICD-10, positive AMA results, death certificates</td>
<td>I + IIb + III or I + IIb</td>
<td>3.22</td>
<td>33.46</td>
<td>8%</td>
</tr>
<tr>
<td>Metcalf [26] Newcastle upon Tyne, United Kingdom</td>
<td>1987 - 1994</td>
<td>160</td>
<td>285,310</td>
<td>personal registry physicians, ICD-10, positive AMA results, death certificates, liver pathology reports</td>
<td>I + IIb + III or I + IIb</td>
<td>5.8</td>
<td>39.2</td>
<td>10%</td>
</tr>
<tr>
<td>Byron [35] Winnipeg, Manitoba, Canada</td>
<td>1987 - 1994</td>
<td>52</td>
<td>650,000</td>
<td>all clinical records referral center</td>
<td>I + IIb + IV</td>
<td>N/A</td>
<td>8</td>
<td>N/A</td>
</tr>
<tr>
<td>Kim [36] Olmsted County, United States</td>
<td>1975 – 1995</td>
<td>46</td>
<td>?</td>
<td>medical record database, pathology database, positive AMA results</td>
<td>I + II or I + III</td>
<td>2.7</td>
<td>40.2</td>
<td>11%</td>
</tr>
<tr>
<td>Boberg [27] Oslo, Norway</td>
<td>1986 - 1995</td>
<td>21</td>
<td>130,000</td>
<td>prospective registration</td>
<td>I + II or I + III + IV</td>
<td>1.6 (1.0-2.5)</td>
<td>14.6 (7.1-11.1)</td>
<td>24%</td>
</tr>
<tr>
<td>Rauttlainen [28] Finland</td>
<td>1988 - 1999</td>
<td>545</td>
<td>2,972,189</td>
<td>ICD-9, ICD-10, pathology registry, personal registry physicians, discharge database transplantation unit</td>
<td>I + IIb + III</td>
<td>1.7 (1.5-2.0)</td>
<td>18.0 (7.2-18.9)</td>
<td>13%</td>
</tr>
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</table>
## Table 2.2 Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Period</th>
<th>No. of patients</th>
<th>Population</th>
<th>Case-Finding</th>
<th>Case-Ascertainment</th>
<th>Incidence per 100,000 (95% CI)</th>
<th>Prevalence per 100,000 (95% CI)</th>
<th>Male</th>
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</thead>
<tbody>
<tr>
<td>Hurlburt [37]</td>
<td>1984 - 2000</td>
<td>18</td>
<td>100,312</td>
<td>All clinical records, ICD-9</td>
<td>I + IIb + III</td>
<td>N/A</td>
<td>16</td>
<td>0%</td>
</tr>
<tr>
<td>Eaton [29]</td>
<td>1977 - 2001</td>
<td>666</td>
<td>5,472,032</td>
<td>ICD-8, ICD-10</td>
<td>N/A</td>
<td>12</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Pla [30] Sabadell, Spain</td>
<td>1990 - 2002</td>
<td>87</td>
<td>389,758</td>
<td>Prospective registration, personal registry physicians, pathology registry, positive AMA results, hospital medical record system</td>
<td>I + II + III</td>
<td>1.72</td>
<td>19.5</td>
<td>3%</td>
</tr>
<tr>
<td>Sood [46] Victoria, Australia</td>
<td>1990 - 2002</td>
<td>249</td>
<td>4,880,000</td>
<td>Personal registry physicians, database liver transplantation unit, medical record search, positive AMA results</td>
<td>I + IIb + III</td>
<td>N/A</td>
<td>5.1</td>
<td>11%</td>
</tr>
<tr>
<td>Myers [38] Alberta, Canada</td>
<td>1996 - 2002</td>
<td>137</td>
<td>1,100,000</td>
<td>Physician claims database, inpatient discharge abstract database, ambulatory care classification system database</td>
<td>I + IIb + III</td>
<td>3.03</td>
<td>22.7</td>
<td>17%</td>
</tr>
<tr>
<td>Delgado [42] Southern Israel</td>
<td>1993 - 2004</td>
<td>47</td>
<td>826,000</td>
<td>Personal registry physicians, liver transplantation registry, ICD-9, positive AMA results, pathology registry, autopsy certificates</td>
<td>I + II + III</td>
<td>N/A</td>
<td>5.5</td>
<td>0%</td>
</tr>
<tr>
<td>Chong [43] Brunei</td>
<td>2007</td>
<td>10</td>
<td>390,000</td>
<td>Hepatology clinics register, pathology register, pharmacy database</td>
<td>I + IIb + III</td>
<td>1.0</td>
<td>2.6</td>
<td>0%</td>
</tr>
</tbody>
</table>

Studies fulfilling all quality criteria regarding 1) definition of studied population; 2) case-finding method and 3) case-ascertainment criteria are highlighted in grey.

\*case-ascertainment criteria: I: AMA; IIa: serum AP \( \geq 6 \) months; IIb: cholestatic liver parameters; III: liver biopsy; IV: IgM \( \uparrow \). AMA: antimitochondrial antibodies; ICD: International Classification of Diseases.
2.3. Studies of good quality are highlighted in table 2.1 and 2.2 and incidence and prevalence rates are shown in figures 2.2 and 2.3.

**Figure 2.2** Incidence of primary sclerosing cholangitis and primary biliary cirrhosis (considering high quality studies only).

**Figure 2.3** Prevalence of primary sclerosing cholangitis and primary biliary cirrhosis. (considering high quality studies only). *No PSC-patients during a 17-year study period.*

PSC

Eleven studies on the epidemiology of PSC from 1984 till 2005 were identified of which four fulfilled quality criteria for both case-ascertainment and case-finding. Three were performed in North America between 1976 and 2005, reporting incidence rates ranging from 0 to 0.92 per 100,000 inhabitants per year. In Alaska no PSC patients were identified between 1984 and 2000. In Canada 49 PSC patients were diagnosed in a 5-yr period in a population of 1,112,521 corresponding with an incidence rate of 0.92 per 100,000 inhabitants per year. One prospective population based study from Norway included 17 newly diagnosed PSC patients in a 10-year period between 1986 and 1995 resulting in an incidence rate of 1.31 per 100,000, still the highest incidence rate for PSC found world-wide.

Eight studies reported the proportion of concomitant IBD in PSC-patients ranging from 20% in Singapore up to 76% in Sweden. When combining studies that met all quality criteria, the average proportion of IBD in PSC-patients was 70% (67-73%). Temporal trends in PSC incidence were reported in four studies, all of which demonstrated increasing incidence rates in time.

PBC

Twenty-four studies describing incidence and/or prevalence rates between 1972 and 2007 for PBC were identified. When considering studies of good quality only, the lowest and highest incidence rates for PBC were both found in Newcastle Upon Tyne, United Kingdom, 0.9 per 100,000 inhabitants per year in 1977 and 5.8 per 100,000 inhabitants per year in 1994 respectively. The highest prevalence rate for PBC of 40.2 per 100,000 age- and sex matched inhabitants was found in 1995 in Olmsted County, Minnesota, USA. When combining studies, the mean proportion of female patients was 92% (76-100%). All eight studies depicting yearly prevalence rates for several consecutive years reported increased prevalence rates in time (fig 2.4).
DISCUSSION

This systematic review yielded a wide range in incidence and prevalence rates as well as study quality for PSC and PBC in Europe, North America, Asia and Australia. The incidence and prevalence rates for PSC range from 0.1-1.3 per 100,000 inhabitants/year and 0.1-16.2 per 100,000 inhabitants respectively. In case of PBC incidence rates range from 0.33-5.8 per 100,000 inhabitants/year and prevalence rates range from 1.91-40.2 per 100,000 inhabitants.

Several causes may underlie the differences found. Improvement of diagnostic tools, increasing disease awareness and digitalized patient registration likely contributed to the rising incidence and prevalence rates. In 1970 gastroenterologists were able for the first time to successfully cannulate the papilla of Vater and selectively intubate the ducts using a duodenoscope with omnidirectional angulation, the first ERCP. A disadvantage of ERCP is the risk of pancreatitis, bleeding or perforation. More than 20 years after the introduction of ERCP, MRCP was introduced making it possible to visualize the intra and extra hepatic ducts without the risks associated with ERCP. The introduction of MRCP lowered the threshold for diagnostic imaging and may have resulted in a higher frequency of cholangiographies diagnosing PSC. In 1965 antimitochondrial antibodies were identified as the most important serological marker for PBC. At that time there was no effective treatment available, but that changed in 1982 when the first trials were initiated administering UDCA at a dose of 13-15 mg/kg daily with good result. Improvement of diagnostic tools and disposition of therapeutic modalities likely play a role in increasing prevalence over time, but may also contribute to global differences since these tools and therapies are not equally distributed around the world.

The introduction of computers in healthcare has been a big leap forward in clinical epidemiological research. Case-finding became easier and more accurate after the introduction of digitalized laboratory and pathology databases. Although some studies in the seventies and eighties seemed well performed, the increase in incidence and prevalence rates, especially for PBC, is in all probability partly attributable to a more exhaustive case-finding strategy using computer databases. The method stated by Metcalf and James already published in 1997 is an excellent example of a meticulous case-finding strategy and has set a standard for subsequent studies. These guidelines include: stringent case inclusion criteria; definition of date of disease onset; well defined study period, area and population; multiple case finding methods and rigorous tracing of all possible cases. Temporal trends may partly be explained by these technological developments and case-finding strategies, yet increasing incidence and prevalence rates in time are even observed within studies. Other factors as discussed below, may play a role in increasing incidence and prevalence rates and geographical differences.

PSC

Although true population-based studies are lacking for PSC, two factors seem to play a significant role in the global distribution of the disease: a variable frequency of IBD around the world and differences in HLA-susceptibility among ethnic groups causing population differences. A recent review combining 47 studies concerning the epidemiology of Crohn’s disease showed a wide variety in incidence and prevalence rates with the highest numbers found in Northern Europe, New Zealand and North America, and lowest numbers in South America, Africa and Asia. Recently, a large PSC cohort listed for liver transplantation in the United States was clinically and genetically investigated. The authors were able to demonstrate that the risk of being listed for liver transplantation is significantly associated with ancestral origin and that phenotype differences in PSC exist across ethnicities.

Based on these reports genetic background seems to play a significant role in the etiology and global distribution of the disease. Unfortunately no population based epidemiological studies were performed in Africa or Asia. One study from Singapore falls short in proper case-finding method, hence it is difficult to draw conclusions.

Three out of four of the highest scoring studies were performed in North America. An outlier among these well-conducted studies in North America is a study published in 2002. Clinical records of all cases of autoimmune liver disease at the Alaska Native Medical Center from 1983 till June 2000 were reviewed. Only one referral center in a population of 100,312 provides a solid foundation for an epidemiological study, even though the authors estimate that 10-20% of Alaska natives seek medical care outside the health care delivery system. Strikingly, no PSC patients were found in a seventeen year period. A possible explanation may be the low incidence of inflammatory bowel disease in this population consisting of Eskimo’s, Aleuts and Indians.

With a catchment area of 19,230,000 inhabitants, the study of Escorsell et al. in Spain between 1984 and 1988 is the largest ever conducted. Unfortunately, the case-finding and case-ascertainment method based on a questionnaire sent to gastroenterologists and hepatologists is insufficient for population-based epidemiology.

Recently a systematic review and meta-analysis of the incidence of PSC has been published. Six population based studies form North America and Europe resulted in a combined incidence rate of 1.0 (0.82-1.17) per 100,000 inhabitants. Incidence rates did not differ when stratified for continent. However, the study from the Alaska Native Medical Center was not included in this analysis.
PBC

Between 1972 and 2007, 23 articles have been published describing incidence and prevalence of PBC. Since the introduction of the guidelines for proper epidemiological studies by Metcalf and James in 1997 the quality of studies improved. The highest incidence and prevalence rates to date have been found in Olmsted County, USA, and Newcastle upon Tyne, UK, pointing towards possible geographic or genetic risk factors. However, until 2005 no studies were performed outside the Western World. In 2005 the first study from the Middle East was published identifying 47 women in Southern Israel resulting in an overall prevalence rate of 5.5 per 100,000 inhabitants, a 7-fold lower prevalence rate compared to the UK and USA. Five years later an even lower prevalence rate was found in Brunei Darussalam, Southeast Asia. Ten patients were identified in a catchment area of 390,000 inhabitants. Strikingly, the prevalence rate in the Chinese population was almost twice as high as in the Malay population (4.1 per 100,000 and 2.3 per 100,000 respectively), though the small number of patients is a limitation of the study. Notable differences in sex ratio were found. At present, it remains unclear whether there is a true variation in sex ratio among populations of different geographical areas with different ethnic backgrounds or whether this is a consequence of varying study quality.

The current hypothesis regarding etiology is that PBC is a complex genetic autoimmune disease, meaning that a combination of genetic susceptibility and environmental factors trigger disease. Besides infectious and lifestyle factors several environmental triggers for PBC have been suggested in the last thirty years and these may partly account for differences in geographical distribution. Triger published a study in 1980 revealing a cluster of PBC patients in Sheffield, UK. Almost all patients in this cluster received water from a single water source. However, chemical analysis of the water did not unravel a potential trigger. Twenty years later, another study from the UK showed strong variations in geographical distribution of patients in Northeast England, but this distribution could not be explained by geographical or demographic features. In New York a significant association between a cluster of PBC patients and superfund toxic waste sites contaminated with volatile aromatic hydrocarbons and trichloroethylene was identified, supporting the hypothesis that environmental toxins play a role in the development of PBC. In conclusion, incidence and prevalence rates of both PSC and PBC vary widely and seem to be increasing. True population based epidemiological studies are scarce, especially in PSC, so it is unclear whether these are true variations or due to methodological differences. Proper worldwide epidemiological data may help identifying etiologic factors for these complex diseases. Hence, large population-based studies combining meticulous case-finding and case-ascertainment strategies as stated by Metcalf and James are necessary and may provide clues as to possible genetic background and environmental risk factors for these chronic cholestatic diseases.
REFERENCES


Population-based epidemiology, malignancy risk and outcome of primary sclerosing cholangitis

Kirsten Boonstra, Rinse K. Weersma, Karel J. van Erpecum, Erik A. Rauws, B.W. Marcel Spanier, Alexander C. Poen, Karin M. van Nieuwkerk, Joost P. Drenth, Ben J. Witteman, Hans A. Tuynman, Anton H. Naber, Paul J. Kingma, Henk R. van Buuren, Bart van Hoek, Frank P. Vleggaar, Nan van Geloven, Ulrich Beuers, Cyriel Y. Ponsioen, on behalf of the Epi PSC PBC study group
ABSTRACT

— Background
Extensive population-based studies are much needed to accurately establish the epidemiology and disease course in patients with primary sclerosing cholangitis (PSC).

— Methods
We aimed to obtain population-based prevalence and incidence figures, insight in disease course with regard to survival, liver transplantation and occurrence of malignancies, as well as risk factors thereof. Four independent hospital databases were searched in 44 hospitals in a large geographically defined area of the Netherlands, comprising 50% of the population. In addition, all PSC patients in the three Dutch liver transplant centers and all inflammatory bowel disease (IBD) patients in the adherence area of a large district hospital were identified. All medical records were reviewed on site verifying diagnosis.

— Results
Five hundred and ninety PSC patients were identified, resulting in an incidence of 0.5 and a point prevalence of 6.0 per 100,000. Median follow up was 92 months. Estimated median survival from diagnosis until liver transplantation or PSC-related death in the entire cohort was 21.2 years, as opposed to 13.2 years in the combined transplant centers cohort (n=422)(p<0.0001). Colorectal carcinoma (CRC) risk was 10-fold increased as compared to ulcerative colitis controls and developed at a much younger age (39 yrs [range 26-64]) compared to IBD controls (59 yrs [range 34-73])(p=0.019). Colonoscopic surveillance was associated with significantly better outcome.

— Conclusion
This study exemplifies that for relatively rare diseases it is paramount to collect observational data from large population-based cohorts, because incidence and prevalence rates of PSC are markedly lower and survival much longer than previously reported. The selection bias-free population-based cohort showed a significantly longer survival compared to the tertiary referral cohort. CRC can develop at an early age, warranting surveillance from time of PSC diagnosis.

INTRODUCTION

Primary sclerosing cholangitis (PSC) is an enigmatic cholestatic liver disease affecting the intra- and extrahepatic bile ducts. PSC is more common in men than in women (2:1) and can occur at any age with a peak incidence around 40. Common symptoms associated with PSC are jaundice, pruritus and upper abdominal discomfort although around 40% of patients are asymptomatic at diagnosis. Multifocal strictures and dilatations of the intra- and/or extra hepatic bile ducts seen on cholangiography are hallmarks of PSC. PSC is strongly associated with inflammatory bowel diseases (IBD) and patients are at increased risk for developing colorectal and biliary malignancies. Disease course is highly variable and there is no treatment available with proven efficacy in halting disease progression other than orthotopic liver transplantation (OLT). In Europe, PSC accounts for 9% of OLT indications. The median survival time until death or liver transplantation is reported to be 12 years.

Most epidemiological studies on PSC are retrospective case-series mainly describing disease course and the association with IBD based on tertiary referral series with their immanent selection bias. Large population-based data on incidence and prevalence as well as on the natural history of PSC are scarce, but very important for proper patient counselling, finding clues to etiology, as well as for health care officials dealing with planning future budgets for OLT, which is currently one of the most expensive treatments available, averaging $120,000.

Here, we describe the largest comprehensive PSC cohort to date in order to obtain proper population-based prevalence and incidence figures, insight in disease course with regard to survival, need for liver transplantation and occurrence of PSC-related malignancies, as well as risk factors thereof.

METHODS

Study design and participants
The protocol was approved by the central Committee for Research Ethics in Utrecht and all 44 local ethics committees of the participating hospitals in the Netherlands (trialregister.nl number, NTR2813). An observational longitudinal cohort study was undertaken between Jan 1, 2008, and Dec 31, 2011. All PSC patients in 44 hospitals from 2000 onwards were identified in a geographically defined area of 6 adjacent provinces comprising 50% of the Dutch population. These 44 hospitals provided care to the entire population of the study area. PSC patients in the three liver transplant centers in the Netherlands (University Medical Centre Groningen, Erasmus Medical Centre Rotterdam, and Leiden University Medical
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Population-based epidemiology, malignancy risk and outcome of PSC

Case-finding and case-ascertainment
Case-finding was performed according to the guidelines of Metcalf and James.13 Four independent hospital databases were searched: 1) PALGA, nation wide network and comprehensive registry of histo- and cytopathology in the Netherlands,13 using diagnosis code liver*biopsy*primary sclerosing cholangitis, 2) Hospital billing system using codes 707 (primary sclerosing cholangitis/primary biliary cirrhosis/autoimmune hepatitis), 954 (primary sclerosing cholangitis/primary biliary cirrhosis) and 943 (autoimmune hepatitis) (ICD-10 code K83.0), 3) Endoscopic retrograde cholangiography (ERC) reports in endoscopy-suite databases, and 4) Personal lists of treating physicians. IBD patients were searched on site using the same 4 data sources. PSC-registries in the three liver transplant centers in the Netherlands and the IBD-registry in the pertaining affiliated large tertiary referral center were checked for missed referrals from the area of interest. All medical records were scrutinized on site by two investigators (KB and CP) for ascertainment of PSC diagnosis. The diagnosis was based on: 1) clinical presentation i.e. pruritus, pain in the right upper abdominal quadrant, fatigue, weight loss, and episodes of fever and/or 2) elevated alkaline phosphatase and gamma-glutamyltransferase, not otherwise explained, 3) presence of characteristic bile duct changes with multifocal strictures and segmental dilatations on ERC or magnetic resonance cholangiography (MRC) and/or 4) liver histology and 5) no evidence for secondary sclerosing cholangitis. When criteria 2, 4 and 5 were fulfilled in the absence of cholangiographic abnormalities on MRC or ERC, cases were diagnosed as small duct PSC.13 Autoimmune hepatitis overlap syndrome (PSC-AIH) is ill-defined. A diagnosis of PSC-AIH was made in patients with a characteristic cholangiogram who in addition met the simplified AIH criteria.13 IBD diagnosis was based on the Lennard-Jones criteria.16

Data collection
At study inclusion and during follow up, apart from demographic characteristics, the following data and endpoints were collected: date and type of PSC diagnosis, date of diagnosis and type of IBD including Montreal classification, date and cause of death, date and type of malignancy, date and type of surgery and cumulative medication use ≥ 6 months for ursodeoxycholic acid (UDCA), thiopurines, and mesalamine during follow up. For non-steroidal anti inflammatory drugs (NSAIDs) including aspirin any prescription use was counted. Date of PSC diagnosis is defined as the date of the diagnostic procedure confirming the diagnosis (ERC, MRC, or liver biopsy). Colonoscopic surveillance was defined as a full-colonoscopy at PSC diagnosis and every 1-2 years after diagnosis in PSC-IBD patients. The occurrence of carcinoma in the cohort was double-checked by linkage of the PSC cohort to PALGA, the nationwide network and registry of histo- and cytopathology in the Netherlands.13

Annual follow-up was obtained by written correspondence from the treating physician. Date of diagnosis was the starting point of follow-up. End of follow up was defined as death, last visit to outpatient clinic or end of the study (January 2012). PSC-related death was defined as death caused by liver disease, cholangiocarcinoma or colorectal carcinoma. Date of death was retrieved from the national death registry. Mid-year, age, and sex-specific population estimates were based on data from the Dutch Central Office for Statistics (http://statline.cbs.nl/statweb). Data on the incidence of malignancies in the general population were retrieved from the Dutch Cancer Registry (www.cijfersoverkanker.nl).

Statistical analyses
Date of diagnosis was defined as the starting point of the disease for all analyses. Cochrane-Armitage test for trend was used to test changes in prevalence and incidence. Kaplan-Meier survival analysis was performed to estimate the cumulative survival. Estimated median survival times were calculated for 1) the combined endpoint PSC-related death or liver transplantation, 2) liver transplantation, and 3) PSC-related death censored at liver transplantation. The overall difference in survival was investigated by the log-rank test. The standardized mortality ratio (SMR) and standardized incidence ratio (SIR) were calculated as the ratios of observed compared with expected number of deaths and malignancies in the study cohort. The expected number of patients was calculated based on the age- and gender-specific mortality and malignancy rates in the general population. When calculating the cumulative risk and SIR for cholangiocarcinoma (CCA), censoring was performed for liver transplantation. In case of dysplasia or CRC, cases were censored at time of colectomy. The extended Cox Model for time-dependent (CCA, CRC, OLT, colectomy) and time-independent variables (Age at diagnosis, gender, PSC type, AIH, IBD, extension colitis, medication) was used for uni- and multivariate analysis of risk factors for endpoints PSC-related death, OLT, CCA and CRC. The assumption of proportional hazards was tested using log minus log survival plots and found valid. Risk factors with a p-value <0.1 in univariate analysis were entered in the multivariate analysis. The Mann–Whitney U-test was performed for comparing continuous data with a non normal distribution. The chi-square test or Fisher’s exact test were used for categorical data. Statistical analyses were performed using SPSS v. 19.0 software (Chicago, IL). P < 0.05 was considered statistically significant.
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RESULTS

Study inclusion

The case-finding strategy yielded 3020 individuals. Of these, 695 were alive on January 1st 2000 and fulfilled the diagnostic criteria for PSC. Reasons for study exclusion are depicted in figure 3.1. Five hundred and ninety PSC patients were resident in the predefined study area between 2000 and 2007 (fig. 3.2). The total number of inhabitants increased during the study period from 7,342,295 in 2000 to 7,758,980 in 2007. The second PSC cohort, accrued from the three Dutch transplantation centers outside the study region yielded 450 cases, of whom 134 (30%) were also present in the population-based cohort. Of these, 422 were alive after January 1 2000 and entered in the comparison. The IBD control cohort comprised 722 cases from a population of 271,000.

Figure 3.1 Flowchart patient inclusion.

Figure 3.2 Map of the Netherlands showing the geographically defined area and place of residence of all 590 PSC patients at diagnosis.

Patient characteristics

Main patient characteristics are shown in table 3.1. The median follow up from diagnosis until death or end of study was 92 months (0-470). Mean age at diagnosis was 38.9 (SD 15.2) years. Among the 590 patients, 375 (64%) were male. Fifty-eight (9%) patients had small duct PSC and 23 (4%) fulfilled the criteria for overlap syndrome with autoimmune hepatitis. In total, 402 (68%) PSC patients were diagnosed with IBD. Overall, 393 (98%) of PSC-IBD patients had ulcerative colitis (UC) or colonic Crohn’s disease (CD) and only 5/78 (6%) CD patients had isolated ileal disease. Ninety-two percent (493/534) of patients were treated with ursodeoxycholic acid (UDCA); 68% (300/438) with mesalamine; 35% (118/333) with thiopurines and 34% (107/317) of patients were using NSAIDs.
Incidence and prevalence

The mean annual incidence between 2000 and 2007 was 0.5 per 100,000 inhabitants; 0.6 in men and 0.4 in women. The age- and gender standardized incidence rates ranged from 0.25 in female adolescents up to 0.93 per 100,000 in men between the ages 40-49 (fig. 3.3). On January 1st 2008 the point prevalence was 6.0 per 100,000 inhabitants; prevalence rates increased significantly over time (p<0.001) (fig. 3.4). Net growth in prevalence was attributable to increase in incidence and not to a decrease in number of deaths (data not shown). In addition, bilirubin levels at diagnosis did not change between 2000 and 2007 (fig. 3.5).

Natural history

During follow up, there were 97 deaths of which 73 (75%) were PSC-related. Sixteen (3%) patients were lost to follow up. The estimated median survival times from diagnosis until the combined endpoint liver transplantation (n=94) or PSC-related death (n=73), until OLT, or until PSC-related death were 21.2, 27.0 and 33.6 years, respectively. When including all deaths in the analysis, the estimated median transplant-free survival was 20.6 years. Because the survival was considerably longer than previously reported, a second cohort was accrued consisting of 450 PSC patients from all three liver transplant centers in the Netherlands to assess the influence of referral bias. Notably, the population-based cohort showed a significantly longer survival from date of diagnosis until the combined endpoint liver transplantation or PSC-related death compared to this tertiary referral centers cohort (21.2 vs. 13.2 years; p<0.0001) (fig. 3.6).

Patients with small duct PSC had a much better survival until PSC-related death or OLT compared with large duct PSC (p=0.019) (fig. 3.7). Concurrence of autoimmune hepatitis (AIH) did not affect transplant-free survival (p=0.58). PSC patients had a four-fold increased risk of mortality...
compared with the general population (SMR 4.2; 95% CI 3.2-5.4). The four most frequent causes of death were cholangiocarcinoma (32%), liver failure (18%), OLT-related complications (9%) and colorectal carcinoma (8%). Ninety-four patients received a liver transplant after median disease duration of 8.1 years (range 0.3-31.3). In multivariate analysis, age at diagnosis, CRC, and CCA were risk factors for the endpoint PSC-related death. NSAID use was associated with a decreased risk for the endpoint liver transplantation (p=0.018) (table 3.2).

CCA occurred in 41/590 (7%) patients of whom 33 died (80%) after a median period of one year (range 0-7). The median age at CCA diagnosis was 47 years (range 21-87) and the median time between PSC diagnosis and CCA was six years (range 0-36). All patients except one had large duct PSC and 27 (66%) had concomitant IBD, mainly UC. There was a 398-fold increased risk for developing CCA in PSC-patients compared with the general population (SIR 398; 95% CI 246-608). Five (12%) patients were diagnosed with PSC and CCA at initial presentation, another six (15%) within the first year, fifteen (37%) between one and ten years, and the remaining fifteen (37%) patients developed a CCA ten or more years after the PSC diagnosis. The cumulative risk of CCA after 10, 20 and 30 years was 6% (95% CI 2-15), 14% (95% CI 4-25), and 20% (95% CI 6-40) respectively (fig. 3.8). Older age at PSC diagnosis was an independent risk factor for the endpoint CCA (HR 1.02; 95% CI 1.00-1.04; p=0.049). The occurrence of CRC was a time-dependent risk factor for CCA (HR 4.57; 95% CI 1.08-19.41; p=0.040).
### Table 3.2 Uni- and multivariate analyses for endpoints PSC-related death and liver transplantation.

<table>
<thead>
<tr>
<th>Endpoint PSC-related death</th>
<th>univariate analysis</th>
<th>multivariate analysis</th>
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<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
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<tr>
<td>Age at diagnosis</td>
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<td>Small duct PSC</td>
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<td>Concurrence of AIH</td>
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<td>Concurrence of IBD</td>
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<td>Extension of colitis</td>
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<td>0.62-3.30</td>
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<tr>
<td>NSAID</td>
<td>0.28</td>
<td>0.08-0.91</td>
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### Endpoint liver transplantation

| Age at diagnosis                      | 1.00    | 0.99-1.02 | 0.67   | 1.01    | 1.00-1.03 | 0.42  |
| Female gender                         | 0.71    | 0.45-1.11 | 0.14   | 0.57    | 0.30-1.06 | 0.074 |
| Small duct PSC                        | 0.28    | 0.07-1.13 | 0.074  | 0.56    | 0.14-2.36 | 0.43  |
| Concurrence of AIH                    | 1.46    | 0.59-3.64 | 0.41   | not in model |
| Concurrence of IBD                    | 1.14    | 0.71-1.83 | 0.58   | not in model |
| Extension of colitis                  | 1.43    | 0.73-2.78 | 0.29   | not in model |
| Colectomy                            | 1.22    | 0.68-2.20 | 0.51   | not in model |
| UDCA                                 | 1.60    | 0.74-3.46 | 0.24   | not in model |
| 5-ASA                                | 0.60    | 0.38-0.95 | 0.029  | 0.77    | 0.55-1.28 | 0.31  |
| NSAID                                | 0.44    | 0.23-0.85 | 0.014  | 0.46    | 0.22-0.94 | 0.033 |

The extended Cox model for time-dependent and time-independent variables was used for uni- and multivariate analysis of risk factors for endpoints PSC-related death, OLT, CCA and CRC. Risk factors with a P-value <0.1 in univariate analysis were entered in the multivariate analysis.

Figure 3.8 Cumulative risk of cholangiocarcinoma (CCA) and high-grade colon dysplasia or colorectal carcinoma (CRC) in PSC patients. 22 patients with proctocolectomy or CRC before diagnosis of PSC were excluded from the analysis.

Twenty out of 590 PSC patients (3%) developed CRC of whom ten died; eight CRC-related and two CCA-related. The median age at CRC diagnosis was 41 (range 26-64) (fig. 3.9). The cumulative risk of high-grade dysplasia or CRC after 10, 20 and 30 years since PSC diagnosis was 3%, 7% and 13%, respectively (fig. 3.8). All CRC patients had large duct PSC. There was a five-fold increased risk for developing CRC in all PSC patients compared with the age- and gender-matched general population (SIR 5.0; 95% CI 2.02-10.3). IBD was present in 19 (95%) patients (18 UC, 1 CD) and the median time between IBD diagnosis and CRC was 15 years (range 0-35). One CRC patient had no endoscopic or histologic signs of IBD after 14 years of follow up. Seven (7/722) IBD control patients developed CRC (SIR 1.2; 95% CI 0.3-3.0) after a median time span of 4 years (range 0-19) after diagnosis (four UC, two CD, and one IBD-U). There was a nine-fold increased risk for developing CRC in PSC-UC patients compared with the age- and gender-matched population (SIR 8.6; 95% CI 3.5-17.7) and a ten-fold increased risk compared to UC controls (ratio of SIRs 9.8; 95% CI 1.9-96.6). Strikingly, CRC occurred at a much younger age in PSC-IBD patients (median age 39 [range 26-64]) compared to IBD controls (59 [range 34-73] (p=0.019)(fig. 3.9). For PSC-IBD patients, the cumulative risk of CRC after 10, 20, and 30 years since IBD diagnosis was 1% (95% CI 0-15) 6% (95% CI 1-22), 13% (95% CI 2-37), respectively (fig. 3.10). Surveillance colonoscopies had been performed prior to CRC diagnosis in 53% of PSC-IBD patients with CRC. When combining PSC-CRC...
DISCUSSION

We here present by far the largest population-based study of PSC with the longest follow up to date. We report markedly lower incidence and prevalence rates of 0.5 per 100,000 and 6.0 per 100,000 inhabitants, respectively, compared to previous studies.\textsuperscript{17–20} Prevalence is rising with stable mortality over time (data not shown), which is in concordance with a recent systematic review and perhaps explained by the rising incidence of IBD in North-West Europe.\textsuperscript{17,21,22} Increased incidence could not be explained by earlier diagnosis, because bilirubin levels at diagnosis remained stable over time and newer diagnostic modalities were not introduced in the observed period. The difference with previous studies points to the importance of performing large population-based studies in relative rare diseases in order to obtain accurate observational data.

Most epidemiological studies on PSC have been performed in specialized centers, prone for selection and referral bias. Between 1984 and 2012 only five small high quality population-based studies have been performed reporting incidence rates ranging from 0 to 1.6 per 100,000 inhabitants based on a collective number of only 96 PSC patients.\textsuperscript{17–20,23,24} When studying a rare disease in only a few 100,000 two or three cases more or less can make a patients from the population-based and transplantation centers cohorts 50% (9/18) of the non-surveilled patients and 16% (3/19) of the patients who received regular surveillance colonoscopies died of CRC ($p=0.038$) (fig. 3.11).
The estimated median survival until liver transplantation or PSC-related death found in the present study is much longer compared to previous reports. When comparing studies on the natural history of PSC several factors play an important role in the survival analysis: starting point of the disease, the definition of endpoints, and proportion of patients that underwent liver transplantation. The introduction of MRC as a non-invasive diagnostic tool, physician awareness and an increase in routine laboratory blood tests may have resulted in an earlier detection of the disease over time. The vast majority of authors use time of diagnosis as starting point for follow-up.\textsuperscript{12,15,19} The definition of PSC-related death differs between studies. Some studies used death from any cause as endpoint for Kaplan-Meier analysis.\textsuperscript{1,17,29} When we included all deaths in the analysis, the estimated median survival until liver transplantation or death was 20.6 years. In three of the largest studies, death from liver disease was used as endpoint, excluding death from CRC.\textsuperscript{1,2} Considering the increased risk for CRC in PSC, we included CRC-related death in the survival analysis. However, when the 8 CRC-related deaths were excluded from analysis, the estimated median survival was 21.3 years, a difference of only 0.1 year. Median transplant-free survival estimates have been reported in the last 25 years ranging from 9.3 years up to 18 years.\textsuperscript{1,15,17,29,30} However, these figures were all based on cohorts from large referral or liver transplantation centers, which may constitute another important bias. As in our cohort, in all but one of these studies the starting point for follow-up was date of diagnosis. In a recent single center cohort from Germany reporting a transplant-free median survival of 9.3 years, 40% of patients had undergone OLT, versus 16% in our cohort.\textsuperscript{9} In the present study we demonstrate the impact of selection bias by comparing our general population-based cohort with a cohort comprising all PSC patients, matched for the population-based cohort inclusion criteria, alive after January 1 2000, as well as >=18 years at inclusion (n=422) from the three transplantation centers in the Netherlands. This contemporary tertiary referral cohort which had a 30% overlap with the population based cohort but immanent by its referral nature not matched for baseline characteristics (table 3.1) showed an estimated median survival until liver transplantation or PSC-related death of 13.2 years. This figure is in concordance with the literature, but almost 40% shorter than patients included in our population-based cohort. These findings highlight the influence of selection bias and hence again the importance of performing large population-based studies in order to get a reliable estimate of the natural history of a disease.

The high preponderance of UDCA treatment in our cohort precluded investigating the influence of this drug on disease course. However, UDCA was also routinely prescribed to most patients in the transplant centers cohort. Moreover, the three largest randomized studies have not been able to demonstrate a benefit of UDCA in halting disease progression, although this does not exclude an effect in early stage PSC.\textsuperscript{26-28} The present study describes for the first time a negative association between NSAID use and liver transplantation. Most NSAIDs inhibit cyclooxygenase (COX), an enzyme that converts arachidonic acid into prostaglandin. There are some reports that COX-2 is over-expressed in liver cirrhosis from HBV and HCV, as well as in various malignancies including HCC.\textsuperscript{29,30} Interestingly, Chávex et al.\textsuperscript{31} showed a beneficial effect of acetyl salicylic acid and ibuprofen in an experimental rat model of liver fibrosis. The NSAIDs used in this model inhibited oxidative stress, COX-2 activity and NFκB translocation to the nucleus. NFκB plays a central role in the inflammation-fibrosis-cancer axis in the liver by inducing TNF-α and IL-6 activating hepatic stellate cells to produce profibrogenic factors.\textsuperscript{32} Recently, a large prospective study showed that NSAIDs reduced the risk of death due to chronic liver disease, even in individuals who only used NSAIDs less than 2-3 times per month.\textsuperscript{33} Our finding clearly deserves further investigation.

Patients with PSC are at highly increased risk for developing CCA, which has a very dismal prognosis. Developing surveillance tools for CCA is one of the prime unmet needs in PSC. CCA was diagnosed within the first year after diagnosis in 2% of PSC patients. Thereafter, the cumulative risk of developing CCA gradually rose to an estimated 20% after 30 years, arguing against a distinct pro-carcinogenic sub-phenotype with a high incidence of early CCA.\textsuperscript{25,26,34,35} Moreover, it is well-known that PSC in retrospect can have a long subclinical time lag until diagnosis of up to 38 years (median 4.3 years).\textsuperscript{2}

Colorectal carcinoma is reported to occur more frequently in IBD patients than in the general population.\textsuperscript{36} We observed that the CRC risk in a population-based IBD cohort is equal to the general population. In accordance with our findings, a study from Denmark showed that the CRC risk in UC patients has declined in the last 30 years and no longer exceeds that of the general population. However this excluded UC patients diagnosed at a young age, with a long disease duration or concomitant PSC.\textsuperscript{37} PSC is an additional and independent risk factor for the development of CRC in UC patients, although low absolute numbers often hamper standardized incidence ratio analysis, emphasizing the need for large population-based cohorts.\textsuperscript{38-40} Our study confirms the increased risk for CRC with a five-fold increase in all PSC patients compared with the general population and a ten-fold increase in PSC-UC patients.
compared to UC alone. Furthermore, CRC develops on average more than 20 years earlier compared to IBD patients and the general population and ranks among the top four causes of death in PSC patients, corroborating current guidelines to start surveillance every 1-2 years from start of diagnosis.²⁴,²⁵ PSC-IBD patients whose CRC was discovered in a surveillance programme showed a significantly better outcome.

Limitations of this study lie in its retrospective nature with its immanent risk of incomplete datasets. However, the proportion of patients lost to follow-up was only 3%, and because we focused on rather easy to appreciate endpoints such as OLT, death, and the occurrence of cholangiocarcinoma, we feel that the chance of missing these data is limited. Also, true population-based figures are impossible to obtain since PSC is a hospital diagnosis. Although we have extensively searched all hospitals in the predefined geographic area and the potential tertiary referral hospitals, there is always a chance of missing some cases and therefore slightly underestimating incidence and prevalence. The capture recapture method, which can correct for this could not be applied, since three out of four data sources were local databases. Not all PSC patients without clinical signs of bowel disease have undergone a screening colonoscopy in the past, which may give an underestimation of concurrent subclinical IBD.

In conclusion, for accurate assessment of the epidemiology and natural history of uncommon diseases like PSC extensive population-based studies adhering to rigorous case-finding guidelines such as the Metcalf and James criteria²⁷ are indispensable. We found that the incidence and prevalence rates of PSC are markedly lower and survival much longer than previously reported. Notably, the population-based cohort showed a significantly longer survival until liver transplantation or PSC-related death compared to a tertiary referral centers cohort, exemplifying the effect of selection bias. The incidence of cholangiocarcinoma in PSC is high and colorectal carcinoma can develop at a young age warranting endoscopic surveillance from the time of diagnosis.

REFERENCES


Primary sclerosing cholangitis is associated with a distinct phenotype of inflammatory bowel disease

Kirsten Boonstra, Karel J. van Erpecum, Karin M. van Nieuwkerk, Joost P. Drenth, Alexander C. Poen, Ben J. Witteman, Hans A. Tuynman, Ulrich Beuers, Cyriel Y. Ponsioen
**ABSTRACT**

— **Background**

PSC is strongly associated with IBD. The aim of this study was to assess the IBD phenotype associated with PSC in a large well phenotyped population-based PSC cohort using endoscopic and histopathologic criteria.

— **Methods**

PSC cases were identified and ascertained, fulfilling established criteria in 39 hospitals in a geographically defined region of the Netherlands. IBD location was recorded according to the Montreal classification. As this classification does not consider segmental inflammation, backwash ileitis or rectal sparing, an additional subgroup analysis was performed in 80 cases and 80 age- and sex-matched IBD controls, reviewing all endoscopy and pathology reports filed between 2000 and 2010.

— **Results**

380 (66%) of a total of 579 PSC patients had coexistent IBD, mainly UC (75%). 207 (83%) of the PSC-UC patients had a pancolitis, 32 (13%) a left sided colitis and 9 (4%) a proctitis only. Seventy (95%) PSC-CD patients had an (ileo)colitis and 4 (5%) ileitis only. In the subgroup analysis 53 (66%) PSC-UC patients were identified, 24 (30%) PSC-CD patients, and 3 (4%) PSC-IBD-U patients. Fifty (94%) PSC-UC patients had a pancolitis, compared to 32 (62%) matched UC controls (p<0.001). Left sided colitis was seen in 16 (31%) UC controls and in one PSC-UC patient (p<0.001). Backwash ileitis and rectal sparing were rare findings (<10%) in the cohorts under study.

— **Conclusion**

Inflammatory bowel disease in PSC patients represents a distinct phenotype in that pancolitis is observed in 94% of PSC-UC and colitis in 96% of PSC-CD patients. Backwash ileitis and rectal sparing were rare findings in the PSC-UC patients.

**INTRODUCTION**

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease affecting the intra- and extrahepatic bile ducts leading to cirrhosis and liver failure with median survival rates until death or liver transplantation from 12 to 18 years.¹ PSC is more common in men than in women (2:1) and can occur at any age with a peak incidence around 40 years. PSC is strongly associated with inflammatory bowel diseases (IBD), often classified as ulcerative colitis (UC), with a prevalence of IBD in PSC patients ranging from 67% to 73%.² IBD associated with PSC (PSC-IBD) is reported to represent a distinct IBD phenotype characterized by pancolitis, rectal sparing and backwash ileitis, but this has so far not been confirmed in large well-phenotyped cohorts.³,⁴ The aim of this study was to assess the IBD phenotype associated with PSC in a large thoroughly ascertained and phenotyped PSC cohort using endoscopic and histopathologic criteria.

**PATIENTS AND METHODS**

**Patients**

PSC cases were recruited from 39 hospitals in a geographically defined region comprising 50% of the Dutch population (approximately 8 million inhabitants). Case-finding and case-ascertainment was performed according to Metcalf and James.⁵ Case-finding was performed through use of four independent hospital databases; PALGA nationwide network and registry of histo- and cytopathology reports, hospital billing system, ERCP-reports in endoscopy-suite databases and personal registry of physicians. All medical records were scrutinized by an expert panel evaluating the diagnosis of PSC and IBD, as well as classifying the disease phenotype. This expert panel consisted of an experienced gastroenterologist (CP) and a dedicated research-fellow specialized in cholestatic liver diseases and IBD (KB). The diagnosis of PSC was based on presence of typical cholangiographic abnormalities of PSC and compatible clinical, biochemical, and hepatic histologic findings. The diagnosis of IBD was based on endoscopic and histopathologic criteria. The study was approved by the central Committee for Research Ethics in Utrecht and all 39 local ethics committees of the participating hospitals in the Netherlands.

**Methods**

IBD location was recorded according to the Montreal classification, i.e. proctitis, left-sided colitis and pancolitis in case of ulcerative colitis and ileitis, colitis and ileocolitis in case of Crohn’s disease (CD) in all PSC-IBD patients in 39 hospitals based on clinical, endoscopy and pathology reports.⁶ Endoscopy reports of 44 PSC-IBD patients could not be retrieved.
and these patients were excluded from analyses. As segmental colitis, backwash ileitis and rectal sparing are not part of the Montreal classification, an additional subgroup analysis was performed in 80 cases and 80 age- and sex-matched IBD controls with at least one complete ileocolonoscopy, reviewing all endoscopy and pathology reports filed between 2000 and 2010 in the Academic Medical Center, Amsterdam and University Medical Center, Utrecht, the Netherlands. Disease location was recorded per segment including the terminal ileum. Backwash ileitis was defined as inflammation seen by endoscopy and histology in the presence of cecal inflammation in ulcerative colitis patients. Rectal sparing was defined as absence of inflammation or significantly less inflammation compared to the sigmoid. Patients using rectal enema therapy within six months prior to colonoscopy were not considered to have true rectal sparing. Follow-up was defined as number of years from date of first diagnosis (PSC or IBD) until dead or inclusion.

Statistical analysis
The Mann-Whitney U test was performed for comparing continuous data with a non-normal distribution. The Chi-squared test or Fisher’s exact test were used for categorical data. P-values less then 0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 16.0 software.

RESULTS
Prevalence of IBD in PSC
579 PSC patients were included, 363 (63%) males and 216 (37%) females. Median follow-up time until dead or inclusion was 15 years (range 0-64). At time of inclusion 380 (66%) patients had coexistent IBD. Of these PSC-IBD patients, 287 (75%) were diagnosed with UC, 78 (21%) with CD and in 15 (4%) cases the IBD remained unspecified as no clear-cut differential diagnosis between UC or CD could be made (IBD-U) (table 4.1). The median age at time of diagnosis of IBD was 30 years (range 1-81). PSC was diagnosed at a median age of 37 years (range 2-83). In most cases the diagnosis of IBD preceded that of PSC (61%) with a median interval of 9 years (range 1–63). In 23% of cases both IBD and PSC were diagnosed in the same year and in a minority (16%) the diagnosis of PSC was established prior to the IBD diagnosis with an interval ranging from 1 up to 32 years.

IBD classification
207 (83%) PSC-UC patients had a pancolitis, 32 (13%) suffered from left-sided colitis and 9 (4%) had proctitis only. Seventy (95%) PSC-CD patients had an (ileo)colitis and 4 (5%) ileitis only (table 4.1).

<table>
<thead>
<tr>
<th>Table 4.1 Demographics and clinical features of PSC-IBD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PSC-IBD patients</strong></td>
</tr>
<tr>
<td><strong>n = 380</strong></td>
</tr>
<tr>
<td><strong>Males [n (%)]</strong></td>
</tr>
<tr>
<td><strong>Small duct PSC [n (%)]</strong></td>
</tr>
<tr>
<td><strong>Age at diagnosis PSC (years) [median (range)]</strong></td>
</tr>
<tr>
<td><strong>Age at diagnosis IBD (years) [median (range)]</strong></td>
</tr>
<tr>
<td><strong>UC [n (%)]</strong></td>
</tr>
<tr>
<td>**E1 proctitis [n (%)]</td>
</tr>
<tr>
<td>**E2 left-sided colitis [n (%)]</td>
</tr>
<tr>
<td>**E3 pancolitis [n (%)]</td>
</tr>
<tr>
<td><strong>Unknown [n]</strong></td>
</tr>
<tr>
<td><strong>CD [n (%)]</strong></td>
</tr>
<tr>
<td>**L1 ileitis [n (%)]</td>
</tr>
<tr>
<td>**L2 colitis [n (%)]</td>
</tr>
<tr>
<td>**L3 ileocolitis [n (%)]</td>
</tr>
<tr>
<td><strong>Unknown [n]</strong></td>
</tr>
<tr>
<td>**IBD-U [n (%)]</td>
</tr>
<tr>
<td>**E1 proctitis [n (%)]</td>
</tr>
<tr>
<td>**E2 left-sided colitis [n (%)]</td>
</tr>
<tr>
<td>**E3 pancolitis [n (%)]</td>
</tr>
<tr>
<td><strong>Unknown [n]</strong></td>
</tr>
<tr>
<td>**IBD preceded PSC [n (%)]</td>
</tr>
<tr>
<td><strong>Time interval between diagnosis IBD and PSC (years) [median (range)]</strong></td>
</tr>
<tr>
<td>**PSC preceded IBD [n (%)]</td>
</tr>
<tr>
<td><strong>Time interval between diagnosis PSC and IBD (years) [median (range)]</strong></td>
</tr>
<tr>
<td>**Concomitant diagnosis of IBD and PSC [n (%)]</td>
</tr>
<tr>
<td><strong>Unknown [n]</strong></td>
</tr>
</tbody>
</table>

PSC, primary sclerosing cholangitis; IBD, inflammatory bowel diseases; UC, ulcerative colitis; CD, Crohn’s disease; IBD-U, IBD-unspecified; unknown, insufficient data.

Subgroup analysis
242 PSC patients were included in the study at the Academic Medical Center, Amsterdam and University Medical Center, Utrecht, the Netherlands. 162 (67%) had a diagnosis of IBD at time of inclusion. 56 PSC-IBD patients received endoscopies elsewhere, in 14 patients
Chapter 4

Chapter 4 PSC is associated with a distinct phenotype of IBD

the ileum was never visualised and 12 patients underwent surgery (ileocecal resection or colectomy) before 2000, leaving 80 suitable PSC-IBD patients for further analysis (fig. 4.1).

![Figure 4.1](Image)

**Figure 4.1** Flow diagram of the included PSC patients (n=242) in the subgroup analysis. Patients without IBD, a colectomy before 2000, no ileoscopy or a colonoscopy performed elsewhere were excluded, leaving 80 PSC-IBD patients for further analysis.

Clinical features

561 colonoscopy reports of 80 PSC-IBD patients and 80 matched IBD controls were reviewed. The median time between IBD diagnosis and study inclusion was 18 years (range 2-59) in PSC-IBD patients and 13 years (range 2-44) in IBD controls (p=0.016). Fifty (94%) PSC-UC patients had pancolitis, compared to 32 (62%) matched UC patients (p<0.001). Left sided colitis was seen in 16 (31%) UC controls and in one (2%) PSC-UC patient (p<0.001). Proctitis was found in 2 PSC-UC patients and 4 UC controls. Backwash ileitis was seen in only 3 (6%) PSC-UC patients and in one (2%) UC control (p=0.618). None of the PSC-CD patients had penetrating disease and only 2 PSC-IBD patients and 5 CD controls developed colonic strictures (p=0.328). Three (4%) PSC patients were diagnosed with IBD-Unspecified. Two PSC-IBD-U patients had pancolitis and one patient had a left-sided colitis compared to three IBD-U controls with pancolitis. One PSC-IBD-U patient and one IBD-U control were diagnosed with backwash ileitis. IBD-U patients were not depicted separately in table 4.2 due to the small number of cases. Overall, 85% of PSC-IBD patients had inflammation of the right hemi-colon compared to 54% of IBD controls (p<0.001) and 78% of PSC-IBD patients had inflammation of the transverse colon compared to 56% of IBD-controls (p=0.004) (table 4.3).

### Table 4.2 Demographics and clinical features of PSC-IBD patients and IBD controls in the subgroup analysis

<table>
<thead>
<tr>
<th></th>
<th>PSC-IBD n=80</th>
<th>IBD n=80</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>57 (71)</td>
<td>57 (71)</td>
<td>1.000</td>
</tr>
<tr>
<td>Age at diagnosis IBD [median (range)]</td>
<td>25 (1-75)</td>
<td>29 (6-58)</td>
<td>0.066</td>
</tr>
<tr>
<td>Disease duration IBD (years) [median (range)]</td>
<td>18 (2-59)</td>
<td>13 (2-44)</td>
<td>0.016</td>
</tr>
<tr>
<td>Age at diagnosis PSC [median (range)]</td>
<td>32 (11-78)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>UC</td>
<td>53 (66)</td>
<td>52 (65)</td>
<td></td>
</tr>
<tr>
<td>E1 proctitis [n (%)]</td>
<td>2 (4)</td>
<td>4 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E2 left-sided colitis [n (%)]</td>
<td>1 (2)</td>
<td>16 (31)</td>
<td></td>
</tr>
<tr>
<td>E3 pancolitis [n (%)]</td>
<td>50 (94)</td>
<td>32 (62)</td>
<td></td>
</tr>
<tr>
<td>Backwash ileitis [n (%)]</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td>0.618</td>
</tr>
<tr>
<td>Rectal sparing [n (%)]</td>
<td>4 (8)</td>
<td>1 (2)</td>
<td>0.363</td>
</tr>
<tr>
<td>No inflammation [n (%)]</td>
<td>2 (50)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Relative sparing [n (%)]</td>
<td>2 (50)</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Bowel surgery [n (%)]</td>
<td>9 (17)</td>
<td>4 (8)</td>
<td>0.236</td>
</tr>
<tr>
<td>Proctocolectomy</td>
<td>8 (15)</td>
<td>3 (6)</td>
<td>1.000</td>
</tr>
<tr>
<td>Ileocecal resection</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Colectomy</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Indication for surgery</td>
<td></td>
<td></td>
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<tr>
<td>Inflammation or stenosis</td>
<td>3 (33)</td>
<td>4 (100)</td>
<td>0.119</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>5 (56)</td>
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<tr>
<td>Carcinoma</td>
<td>1 (11)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminosalicylates</td>
<td>40 (75)</td>
<td>39 (75)</td>
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</tr>
<tr>
<td>Thiopurines</td>
<td>9 (17)</td>
<td>20 (38)</td>
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<td>9 (17)</td>
<td>11 (21)</td>
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<tr>
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<td>1 (2)</td>
<td>6 (12)</td>
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<td>Aminosalicylates monotherapy</td>
<td>30 (57)</td>
<td>15 (29)</td>
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<tr>
<td>No medication</td>
<td>6 (11)</td>
<td>6 (12)</td>
<td>1.000</td>
</tr>
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</table>

Overall, 85% of PSC-IBD patients had inflammation of the right hemi-colon compared to 54% of IBD controls (p<0.001) and 78% of PSC-IBD patients had inflammation of the transverse colon compared to 56% of IBD-controls (p=0.004) (table 4.3).
Table 4.2 Continued

<table>
<thead>
<tr>
<th>Segmental distribution of inflammation in PSC-IBD patients and IBD controls</th>
<th>PSC-IBD</th>
<th>IBD</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>CD</td>
<td>24 (30)</td>
<td>25 (31)</td>
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</tr>
<tr>
<td>L1 ileitis [n (%)]</td>
<td>1 (4)</td>
<td>7 (28)</td>
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</tr>
<tr>
<td>L2 colitis [n (%)]</td>
<td>14 (58)</td>
<td>8 (32)</td>
<td>0.044</td>
</tr>
<tr>
<td>L3 ileocolitis [n (%)]</td>
<td>9 (38)</td>
<td>10 (40)</td>
<td>0.044</td>
</tr>
<tr>
<td>Rectal sparing [n (%)]</td>
<td>3 (13)</td>
<td>2 (8)</td>
<td>0.044</td>
</tr>
<tr>
<td>No inflammation [n (%)]</td>
<td>3 (100)</td>
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<td>0.399</td>
</tr>
<tr>
<td>Less inflammation [n (%)]</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>0.399</td>
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<tr>
<td>Bowel surgery [n (%)]</td>
<td>5 (21)</td>
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<td>Proctocolectomy</td>
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<td>Ileocecal resection</td>
<td>0 (0)</td>
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<tr>
<td>Colectomy</td>
<td>4 (17)</td>
<td>2 (8)</td>
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<tr>
<td>Inflammation or stenosis</td>
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<td>7 (100)</td>
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<td>Dysplasia</td>
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<tr>
<td>Carcinoma</td>
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<td>0.152</td>
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<td>19 (79)</td>
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<td>Anti-TNF</td>
<td>5 (21)</td>
<td>8 (32)</td>
<td>0.377</td>
</tr>
<tr>
<td>Aminosalicylates monotherapy</td>
<td>12 (50)</td>
<td>1 (4)</td>
<td>&lt;0.001</td>
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<tr>
<td>No medication</td>
<td>0 (0)</td>
<td>3 (12)</td>
<td>0.235</td>
</tr>
</tbody>
</table>

PSC, primary sclerosing cholangitis; IBD, inflammatory bowel diseases; UC, ulcerative colitis; CD, Crohn’s disease; IBD-U, IBD-unspecified.

Table 4.3 Segmental distribution of inflammation in PSC-IBD patients and IBD controls

<table>
<thead>
<tr>
<th>Segmental distribution of inflammation in PSC-IBD patients and IBD controls</th>
<th>PSC-IBD</th>
<th>IBD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right hemi-colon [n (%)]</td>
<td>68 (85)</td>
<td>43 (54)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transverse colon [n (%)]</td>
<td>62 (78)</td>
<td>45 (56)</td>
<td>0.004</td>
</tr>
<tr>
<td>Left hemi-colon [n (%)]</td>
<td>69 (86)</td>
<td>68 (85)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

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

DISCUSSION

In this large population-based study, evaluating the IBD phenotype associated with PSC, we found that IBD in PSC patients represents a distinct phenotype in that pancolitis is observed in 94% of PSC-UC patients and colitis in 96% of PSC-CD patients. The right hemi-colon is more often inflamed in PSC-IBD patients compared to matched IBD controls. Backwash ileitis and rectal sparing were rare findings.
Our findings are consistent with the results from a recent study performed in Boston, Massachusetts and St. Louis, Missouri, in 2009. There, the authors found a higher prevalence rate of pancolitis (85%) in the PSC-UC patients compared to a matched UC-control group (45%), but no significant differences in prevalence of backwash ileitis or rectal sparing. In contrast, an association of PSC-IBD with backwash ileitis and rectal sparing was first reported 20 years ago, and later reproduced by Loftus Jr. et al. who found backwash ileitis in 51% (19/37) and rectal sparing in 52% (32/61) of PSC-UC patients. Recently, Jørgensen et al. reported that 84% of 184 prospectively included PSC patients had concomitant IBD. Backwash ileitis was seen in 12% of PSC-UC patients and rectal sparing in 66% of PSC-IBD patients. Some authors have lent credit to backwash ileitis in support of the hypothesis of aberrant homing of gut-homing lymphocytes to the liver in PSC. Previous research has shown increased numbers of gut specific T-cells and chemokines present in the portal infiltrate of livers in a relative early stage of PSC. However, the key chemokine ligand CCL25 has so far only been demonstrated in the small bowel and not in the colon. To accommodate for this, backwash ileitis was put forward. Although we did observe signs of inflammation in ileal biopsies in absence of endoscopic ileitis in 7 (14%) PSC patients with concomitant pancolitis in the subgroup analysis, the prevailing phenotype of concurrent IBD is one of colonic and not ileal involvement. The clinical relevance of histopathologic abnormalities in ileal biopsies in absence of endoscopic ileitis is under debate. In a retrospective analysis of 9785 patients with ileal biopsies, Melton et al. showed that 5% of patients with a normal ileum did have abnormal histopathologic findings and in case of CD patients this percentage was even greater than 10%. The authors concluded that biopsies taken from normal ileum rarely provide relevant information and cannot be recommended. In our subgroup analysis the prevalence of backwash ileitis was low and did not differ between groups. However, we did not perform a power analysis to see a difference in backwash ileitis, due to the difficulty to collect cases, and 56 PSC-IBD patients could not be included in the subgroup analysis due to lack of information. Nevertheless, there was no indication of increased backwash ileitis in our PSC-IBD patients. The relevance of microscopic ileitis in PSC-IBD cases has yet to be elucidated.

In our study, most patients (61%) were first diagnosed with IBD and in some cases it took decades before PSC would surface. In 16% of cases, PSC was diagnosed prior to IBD. Given the median follow-up in our series of 15 years whilst the median time lag between the diagnosis of IBD and PSC successively ranges from 1 to 63 years, and 1 to 32 years vice versa, it could be inferred that the true prevalence of IBD in PSC patients is higher than 66%. The mild and quiescent character of IBD in PSC patients might result in a long subclinical phase. A study from Sweden showed histologic signs of inflammation in 7 out of 9 PSC patients without clinical signs of IBD. Of these 7 PSC-patients developed symptoms one, three and seven years after the first colonoscopy.

In general the inflammatory activity in PSC-IBD patients is low according to the SCCAI (Simple Clinical Colitis Activity Index), as well as according to endoscopic and histopathologic criteria. In line with our findings, various studies have reported a mild course of IBD in PSC patients. Scoring the severity of inflammation was not the main focus of our study, but we did observe that none of the PSC-CD patients had penetrating disease and that PSC-IBD patients in general used less thiopurines or combination therapy. However, these differences in medication use could also in part be due to reticence of treating physicians to prescribe potentially hepatotoxic drugs to patients with a chronic progressive liver disease.

Although the strong association between PSC and IBD is well established, the underlying pathogenesis is not known. Notably, pre-existing UC may worsen after orthotopic liver transplantation (OLT) in 30% of cases and colitis may develop de novo under post-transplant immunosuppressive therapy. Conversely PSC requiring OLT is associated with a milder UC, but no significant differences in prevalence of backwash ileitis or rectal sparing. Our findings are consistent with the results from a recent study performed in Boston, Massachusetts and St. Louis, Missouri, in 2009. The strong association between PSC and IBD is well established, and the underlying pathogenesis is not known. Notably, pre-existing UC may worsen after orthotopic liver transplantation (OLT) in 30% of cases and colitis may develop de novo under post-transplant immunosuppressive therapy. Conversely PSC requiring OLT is associated with a milder UC, but no significant differences in prevalence of backwash ileitis or rectal sparing. However, the key chemokine ligand CCL25 has so far only been demonstrated in the small bowel and not in the colon. To accommodate for this, backwash ileitis was put forward. Although we did observe signs of inflammation in ileal biopsies in absence of endoscopic ileitis in 7 (14%) PSC patients with concomitant pancolitis in the subgroup analysis, the prevailing phenotype of concurrent IBD is one of colonic and not ileal involvement. The clinical relevance of histopathologic abnormalities in ileal biopsies in absence of endoscopic ileitis is under debate. In a retrospective analysis of 9785 patients with ileal biopsies, Melton et al. showed that 5% of patients with a normal ileum did have abnormal histopathologic findings and in case of CD patients this percentage was even greater than 10%. The authors concluded that biopsies taken from normal ileum rarely provide relevant information and cannot be recommended. In our study, most patients (61%) were first diagnosed with IBD and in some cases it took decades before PSC would surface. In 16% of cases, PSC was diagnosed prior to IBD. Given the median follow-up in our series of 15 years whilst the median time lag between the diagnosis of IBD and PSC successively ranges from 1 to 63 years, and 1 to 32 years vice versa, it could be inferred that the true prevalence of IBD in PSC patients is higher than 66%. The mild and quiescent character of IBD in PSC patients might result in a long subclinical phase. A study from Sweden showed histologic signs of inflammation in 7 out of 9 PSC patients without clinical signs of IBD. Of these 7 PSC-patients developed symptoms one, three and seven years after the first colonoscopy.

In general the inflammatory activity in PSC-IBD patients is low according to the SCCAI (Simple Clinical Colitis Activity Index), as well as according to endoscopic and histopathologic criteria. In line with our findings, various studies have reported a mild course of IBD in PSC patients. Scoring the severity of inflammation was not the main focus of our study, but we did observe that none of the PSC-CD patients had penetrating disease and that PSC-IBD patients in general used less thiopurines or combination therapy. However, these differences in medication use could also in part be due to reticence of treating physicians to prescribe potentially hepatotoxic drugs to patients with a chronic progressive liver disease.
possible carcinogens in PSC-IBD. However, reports on the possible effects of the hydrophilic bile acid ursodeoxycholic acid (UDCA) in preventing CRC, by decreasing secondary bile acids, are contradictory.22,23 Almost all PSC patients included in the current study were treated with UDCA and therefore we were unable to study the potential beneficial effect of UDCA. Pardi and colleagues found a 74% reduction in risk for dysplasia or cancer in a post-hoc analysis of a randomized placebo-controlled trial for UDCA-therapy in PSC-UC patients.22 This potential chemopreventive effect of UDCA could not be confirmed by Wolf et al. in a retrospective cohort study of 120 PSC-UC patients. No reduction in risk of developing cancer or dysplasia was found in their PSC-UC patients treated with UDCA.23 Recently, a follow-up study of a randomized controlled trial of high dose UDCA (17-23 mg/kg/day) vs. placebo therapy in PSC patients did not show a difference in frequency of dysplasia or cancer between groups.24

Several studies have been performed evaluating the characteristics of inflammatory bowel diseases associated with PSC in different cohorts ranging from 29 to 184 PSC patients.24,25,26 Yet, none of these studies were population-based. Our PSC cohort is the largest well phenotyped population-based cohort worldwide. Nevertheless, the retrospective design of our study, and the fact that not all PSC patients in the 39 participating hospitals received a colonoscopy in the absence of clinical signs of IBD, presumably resulted in an underestimation of the association between IBD and PSC.

In summary, inflammatory bowel disease in PSC patients represents a distinct phenotype in that pancolitis is observed in 94% of PSC-UC and colitis in 96% of PSC-CD patients. Backwash ileitis and rectal sparing were rare findings in the PSC-UC cohort studied. The increased risk for dysplasia and cancer and the strong correlation between PSC and IBD mandates colonoscopy at diagnosis and annual surveillance.

REFERENCES


Risk factors for primary sclerosing cholangitis


* These authors contributed equally to this work
ABSTRACT

— Background
Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease strongly associated with inflammatory bowel disease (IBD). Little is known about risk factors triggering PSC. The aim of this study was to evaluate geographical distribution, smoking, appendectomy, family history, and seroprevalence of Chlamydia and Reovirus type 3 antibodies in a large group of PSC patients, IBD patients, and healthy controls (HC).

— Methods
PSC patients, IBD patients and HC’s living in a geographically defined area in the Netherlands, filled-out a questionnaire concerning smoking, appendectomy and family history of IBD and liver diseases. Blood samples were collected.

— Results
Ulcerative colitis (UC) patients were more often current (19%) and former smokers (54%) compared to PSC-UC patients (6% and 22%, respectively) (p<0.001). Former smoking was associated with a lower risk of developing PSC in both UC (OR 0.20; 95% CI 0.12 0.34) and Crohn’s disease (CD) patients (OR 0.17; 95% CI 0.08-0.39). Frequency of appendectomy did not differ between PSC and HC, but PSC-UC patients had undergone appendectomy more often than UC patients (13% vs. 6%) (OR 2.24; 95%CI 1.02-4.92). There was no association between positive Reovirus type 3 or Chlamydia antibody titers and risk of PSC. Degree of urbanization was not associated with PSC incidence.

— Conclusion
Smoking is associated with a lower risk of developing PSC, independent of its protective effect in UC. Previous appendectomy is associated with a decreased risk of UC, but not of PSC-UC.

INTRODUCTION
Primary sclerosing cholangitis (PSC) is an enigmatic cholestatic liver disease affecting the intra- and extrahepatic bile ducts. PSC is more common in men than in women (2:1) and can occur at any age with a peak incidence around 40. Disease course is highly variable and there is no treatment available with proven efficacy in halting disease progression other than liver transplantation (LT). PSC is strongly associated with inflammatory bowel diseases (IBD) and patients are at increased risk for developing colorectal and biliary malignancies. Although the strong association between PSC and IBD is well established, the underlying pathogenesis is not known. IBD in PSC patients represents a distinct phenotype in that pancolitis is observed in 94% of PSC-ulcerative colitis (UC) and colitis in 96% of PSC-Crohn’s disease (CD) patients. PSC and IBD are considered to be complex genetic diseases, meaning that a combination of genetic and environmental factors cause disease. In IBD, 163 risk loci have been identified so far and some of these risk loci have also been associated with PSC. However, the magnitude of genetic studies in IBD is ten fold larger than in PSC. First degree relatives of PSC patients run an increased risk of PSC and UC. Besides limited knowledge of the genetic risk factors, little is known about environmental factors triggering disease. Current smoking and previous appendectomy have been shown to protect against developing UC. However, studies on smoking behaviour and appendectomy and the risk of developing PSC are many fold smaller, warranting larger studies. One of the leading hypotheses on the pathogenesis of PSC is that an infectious trigger may cause breakdown of immunological tolerance in bile ducts of genetically susceptible hosts. Limited data has been published concerning the role of bacterial or viral agents in PSC. Reovirus type 3 infection has been linked to PSC for the first time in 1987. Minuk et al. showed that mean titers of anti-Reovirus type 3 were significantly higher in PSC sera compared to controls, yet titers were within the normal range. An association between PSC and previous infection with Chlamydia spp. has been suggested but awaits confirmation in a larger cohort.

The aim of the present case-control study was to evaluate the geographical distribution, smoking behaviour, history of appendectomy, family history, and seroprevalence of Chlamydia spp. and Reovirus type 3 antibodies in a large group of PSC patients, IBD disease controls, and healthy volunteers.
PATIENTS AND METHODS

For this case-control study, patients were recruited from the Epi PSC PBC project; a large population based observational longitudinal cohort study of PSC and PBC in the Netherlands. The case-finding and case-ascertainment methods have been described previously.1

Study design

The protocol was approved by the Central Committee for Research Ethics in Utrecht and all 44 local ethics committees of the participating hospitals in the Netherlands (trialregister.nl, NTR2813). An observational longitudinal cohort study was undertaken between Jan 1, 2008, and Dec 31, 2011. All PSC patients in 44 hospitals from 2000 onwards were identified in a geographically defined area of 6 adjacent provinces comprising 50% of the Dutch population (2000: 7,342,295 – 2007: 7,758,980). This area consists of 169 municipalities subdivided in 5 categories by degree of urbanisation; 1 ≥2500, 2; 1500-2500; 3; 1000-1500, 4; 500-1000, 5; <500 residences per km². The 44 participating hospitals provided care to the entire population of the study area.

Case-finding and case-ascertainment

Case-finding was performed according to the guidelines of Metcalf and James.24 Four independent hospital databases were searched: 1) PALGA, nationwide network and comprehensive registry of histo- and cytopathology in the Netherlands,23 using diagnosis code liver*biopsy*primary sclerosing cholangitis, 2) hospital billing system using codes 707 (primary sclerosing cholangitis/primary biliary cirrhosis/autoimmune hepatitis), 954 (primary sclerosing cholangitis/primary biliary cirrhosis) and 943 (autoimmune hepatitis), 3) endoscopic retrograde cholangiography (ERC) reports in endoscopy-suite databases, and 4) personal lists of treating physicians. All medical records were scrutinized on site by two investigators (KB and CP) for ascertainment of PSC diagnosis. The diagnosis was based on: 1) clinical presentation i.e. pruritus, pain in the right upper abdominal quadrant, fatigue, weight loss, and episodes of fever and/or 2) elevated alkaline phosphatase and gamma-glutamyltransferase, not explained otherwise, 3) presence of characteristic bile duct changes with multifocal strictures and segmental dilatations on ERC or magnetic resonance cholangiography (MRC) and/or 4) liver histology and 5) no evidence for secondary sclerosing cholangitis. When criteria 2, 4 and 5 were fulfilled in the absence of cholangiographic abnormalities on MRC or ERC, cases were diagnosed as small duct PSC.20

For this case-control study, PSC patients with concomitant IBD were subdivided in PSC-UC and PSC-CD subgroups, in which PSC-IBD unspecified (IBD-U) patients were labelled as PSC-UC patients.

Controls

Healthy controls (HC) and IBD patients were randomly recruited from the outpatient clinics of four participating hospitals, equally distributed throughout the study area. Subjects visiting the outpatient clinic without a history of liver disease or autoimmune disease were considered to be healthy controls. These volunteers were healthy individuals accompanying PSC, PBC, and IBD patients tot the outpatient clinic, or visiting the outpatient clinic for regular surveillance of Barrett’s esophagus or colon polyps. IBD diagnosis was based on the Lennard-Jones criteria.20 IBD patients were subdivided in UC and CD patients, IBD-U patients were labelled as UC patients.

Data collection

At inclusion of the Epi PSC PBC project the following data were collected from hospital patient files: date and type of PSC diagnosis, zip code of residence at time of diagnosis, date of diagnosis and type of IBD, smoking behaviour, and date and type of appendectomy. Patients and controls were asked to give informed consent and fill out a questionnaire regarding smoking status (current smoking, former smoking; defined as smoking for more than one year in the past twenty years, and ever smoking; defined as the sum of current smoking and former smoking), history of appendectomy, and first degree family history of auto-immune liver disease and IBD. Data on smoking behaviour and history of appendectomy were combined from both hospital files and questionnaires. Population estimates were retrieved from the Dutch Central Office for Statistics (Centraal Bureau voor Statistiek, Den Haag, the Netherlands; http://statline.cbs.nl).

Serology

Serum samples were obtained from peripheral blood by centrifugation and stored at −20°C. Serum samples of a randomly selected subgroup of PSC patients and IBD controls were tested for IgA and IgG against Chlamydia spp., using Chlamydia-specific IgA and IgG enzyme linked immunosorbent assay (ELISA) (Medac, Hamburg, Germany). Antibody titers against Reovirus type 3 were detected by standard neutralization assay using Vero cells infected with 100 TCID50 Reovirus type 3.

Statistical analysis

Potential risk factors (sex, age, smoking behaviour, appendectomy, family history of IBD and autoimmune liver disease, Chlamydia and Reo virus type 3) for the development of PSC were analyzed by comparing the relative frequencies between PSC patients and healthy controls as well as PSC-IBD patients and IBD controls using an univariable logistic regression model. Only appendectomies performed before PSC diagnosis was established.
were included in the analysis. Relative frequencies were expressed as odds ratio (OR), with corresponding 95% confidence intervals (CI). To differentiate risk factors for the development of PSC from risk factors for IBD, PSC-IBD patients and IBD controls were matched for type of IBD. Potential confounders were checked using multivariable logistic regression modeling. Variables with a p-value <0.1 in univariable analysis were entered in the multivariable analysis. Given the overlap in current, former and ever smoking, only former smoking was considered for multivariable analysis. PSC patients and IBD controls included in the Chlamydia analysis were not subdivided by IBD-type, as groups were too small. Due to correlation between Chlamydia IgA and IgG antibodies, logistic regression analysis was performed separately.

For analysis of geographic distribution, all PSC patients from the Epi PSC PBC cohort were included. PSC incidence was calculated for the sum of municipalities per degree of urbanization, using the mean population between 2000 and 2008. Standardized incidence ratios were calculated to analyse differences between observed and expected incidence of PSC per degree of urbanization. The 95% confidence interval was used to determine if the observed cases were significantly different from the expected cases, in which a range including the integer one denotes no significant difference. Chi-squared test for trend in proportions was used to determine if there was statistical difference of incidence between degrees of urbanization. Statistical analyses were performed using SPSS version 20.0 software (SPSS, Inc., Chicago, IL). p<0.05 was considered statistically significant.

RESULTS

Study inclusion

The case-finding exercise yielded 697 PSC patients. Four-hundred and fifty PSC patients were alive at time of inclusion and were asked to give informed consent and fill out a ten-item questionnaire. In total, 343 PSC patients completed the questionnaire resulting in a response rate of 76%. A total of 254 healthy controls and 404 IBD controls were included, of whom 232 (92%) and 370 (92%) completed the questionnaire, respectively.

Patient characteristics

Patient characteristics are summarized in Table 5.1. Sixty-five percent of PSC patients were male and the median age at time of inclusion was 53 years (IQR 42-63 years). The majority of PSC patients had large duct PSC (89%). Concomitant IBD was present in 218 PSC patients, of whom 168 (77%) had UC, and 50 (23%) CD. Healthy controls had a median age at inclusion of 59 years (IQR 46-69 years) and 46% were male. Of IBD controls, 167 (45%) had UC and 203 (55%) CD, 40% were male, and the median age at inclusion was 47 years (IQR 36-60).

<table>
<thead>
<tr>
<th></th>
<th>PSC</th>
<th>PSC-UC</th>
<th>PSC-CD</th>
<th>HC</th>
<th>UC</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>343</td>
<td>168</td>
<td>50</td>
<td>232</td>
<td>167</td>
<td>203</td>
</tr>
<tr>
<td>Male gender [%]</td>
<td>222 (65)</td>
<td>116 (69)</td>
<td>30 (60)</td>
<td>106 (46)</td>
<td>70 (44)</td>
<td>74 (37)</td>
</tr>
<tr>
<td>Age at inclusion (years) [median (IQR)]</td>
<td>36 (12-71)</td>
<td>35 (12-69)</td>
<td>12 (12-50)</td>
<td>59 (46-69)</td>
<td>51 (38-65)</td>
<td>41 (34-58)</td>
</tr>
<tr>
<td>Small duct PSC [%]</td>
<td>36 (10)</td>
<td>37 (25-44)</td>
<td>37 (25-45)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Age at PSC diagnosis (years) [median (IQR)]</td>
<td>31 (21-44)</td>
<td>28 (6)</td>
<td>28 (6)</td>
<td>31 (21-44)</td>
<td>32 (21-44)</td>
<td>34 (21-44)</td>
</tr>
<tr>
<td>Smoking current [%]</td>
<td>28 (8)</td>
<td>10 (6)</td>
<td>4 (8)</td>
<td>41 (19)</td>
<td>32 (19)</td>
<td>53 (26)</td>
</tr>
<tr>
<td>Smoking former [%]</td>
<td>79 (23)</td>
<td>37 (23)</td>
<td>8 (16)</td>
<td>85 (37)</td>
<td>85 (37)</td>
<td>50 (24)</td>
</tr>
<tr>
<td>First-degree relative with IBD [%]</td>
<td>38 (11)</td>
<td>19 (11)</td>
<td>1 (2)</td>
<td>35 (15)</td>
<td>35 (15)</td>
<td>11 (5)</td>
</tr>
<tr>
<td>First-degree relative with autoimmune liver disease [%]</td>
<td>16 (4)</td>
<td>7 (4)</td>
<td>1 (2)</td>
<td>20 (9)</td>
<td>20 (9)</td>
<td>10 (5)</td>
</tr>
</tbody>
</table>
| PSC = primary sclerosing cholangitis; UC = ulcerative colitis; CD = Crohn’s disease; HC = healthy controls; IBD = inflammatory bowel disease; range = interquartile range; NA = not applicable.

Table 5.1 Patient characteristics.
Risk factors for PSC

**Smoking**

As shown in Table 5.1, there were fewer current smokers (8%) in the PSC group than in the healthy controls (15%), whereas more ever smokers (23%) were found compared to healthy controls (18%). When comparing IBD patients to PSC-IBD patients, UC patients were more often current (19%), former (54%) and ever (54%) smokers compared to PSC-UC patients (6%, 22%, 23% respectively) (p<0.001). Similar results were seen for CD patients compared to PSC-CD patients.

**Appendectomy**

An equal amount of PSC patients and healthy controls underwent appendectomy (13% in both groups). In one PSC patient the appendix was removed as part of an ileo-cecal resection. More PSC-UC patients (13%) than UC controls (6%) had undergone appendectomy (OR 2.24; 95% CI 1.02-4.92). In this subgroup all appendectomies were performed for clinical suspicion of acute appendicitis. When correcting for possible interacting variables using multivariable modeling, the positive association persisted in this subgroup (p=0.046). Less UC patients (6%) than healthy controls (13%) had undergone appendectomy (OR 0.42; 95% CI 0.20-0.89). The vast majority of PSC-UC patients (95%) were diagnosed with UC before PSC. In PSC-UC patients an appendectomy was performed prior to UC diagnosis in 48%, after UC diagnosis and prior to PSC diagnosis in 38% of patients and in 14% of PSC patients an appendectomy was performed at time of UC diagnosis.

**Family history**

The occurrence of PSC among first-degree family members of PSC patients was 1.2% (4/343). UC was present in first-degree relatives of 25 (7.3%) PSC patients. Seventeen (10%) PSC-UC patients and 28 (7%) UC controls had a first-degree family member with IBD. The occurrence of PSC among first-degree family members of PSC patients was 1.2% (4/343). UC controls had a first-degree family member with IBD (OR 0.42; 95% CI 0.95-0.18-0.49) (table 5.2). No differences in IBD prevalence were found between families of PSC-CD and UC patients (16% vs. 17%). The percentages of first-degree relatives with autoimmune liver diseases were similar for PSC-UC patients (1%) and UC controls (1%). Autoimmune liver diseases were more prevalent in families of PSC-CD patients than in families of UC controls, however numbers were very small (2% vs. 0%).

**Chlamydia**

Serum samples of 79 randomly selected PSC patients and 86 IBD controls were tested for the presence of Chlamydia IgA and IgG. 77 PSC patients and 83 IBD controls filled out the questionnaires and eligible for analysis. Sixty-four percent of PSC patients were male, the median age at time of Chlamydia analysis was 54 years (IQR 41-65 years). The IBD controls consisted of 57% UC and 43% CD patients. Forty-one percent were male and the median age at time of Chlamydia analysis was 48 years (IQR 34-62 years). More PSC patients (53%) than IBD controls (36%) had a positive serum Chlamydia IgA titer (OR 2.01; 95% CI 1.07-3.79). Furthermore, 39% of PSC patients showed a positive serum Chlamydia IgG titer, compared to 27% of IBD controls (OR 1.77; 95% CI 0.91-3.46) (table 5.3a-b). Multivariable logistic regression showed no association between a positive serum Chlamydia IgA or IgG titer and PSC (table 5.3a-b).

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**Table 5.2** Univariable and multivariable logistic regression analysis

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Sex</td>
<td>2.1 (1.55-3.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at inclusion</td>
<td>1.03 (1.01-1.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Concomitant IBD</td>
<td>0.003 (0.00-0.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.38 (0.23-0.63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former smoking</td>
<td>0.52 (0.36-0.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ever smoking</td>
<td>0.54 (0.38-0.78)</td>
<td>0.001</td>
</tr>
<tr>
<td>Appendectomy</td>
<td>1.02 (0.64-1.71)</td>
<td>0.868</td>
</tr>
<tr>
<td>First-degree relative with IBD</td>
<td>0.56 (0.29-1.07)</td>
<td>0.077</td>
</tr>
<tr>
<td>First-degree relative with autoimmune liver disease</td>
<td>2.0 (0.18-22.27)</td>
<td>0.57</td>
</tr>
<tr>
<td>PSC vs HC</td>
<td>2.87 (1.84-4.50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSC-UC vs UC</td>
<td>2.79 (1.48-5.26)</td>
<td>0.002</td>
</tr>
<tr>
<td>PSC-CD vs CD</td>
<td>2.79 (1.48-5.26)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

PSC = primary sclerosing cholangitis; HC = healthy controls; UC = ulcerative colitis; CD = Crohn’s disease; IBD = inflammatory bowel disease; OR = odds ratio; CI = confidence interval; NA = not applicable.
### Table 5.3a Univariable and multivariable logistic regression analysis Chlamydia IgA

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>N</td>
<td>77</td>
<td>83</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>49 (64)</td>
<td>34 (41)</td>
</tr>
<tr>
<td>Age at inclusion (years) [median (IQR)]</td>
<td>54 (41-65)</td>
<td>48 (53-56)</td>
</tr>
<tr>
<td>Former smoking [n (%)]</td>
<td>18 (23)</td>
<td>20 (24)</td>
</tr>
<tr>
<td>Appendectomy [n (%)]</td>
<td>12 (16)</td>
<td>12 (15)</td>
</tr>
<tr>
<td>First-degree relative with auto-immune liver disease [n (%)]</td>
<td>3 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Positive Chlamydia IgA titer [n (%)]</td>
<td>41 (53)</td>
<td>30 (36)</td>
</tr>
</tbody>
</table>

PSC = primary sclerosing cholangitis; IBD = inflammatory bowel disease; OR = odds ratio; CI = confidence interval.

### Table 5.3b Univariable and multivariable logistic regression analysis Chlamydia IgG

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>N</td>
<td>77</td>
<td>83</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>49 (64)</td>
<td>34 (41)</td>
</tr>
<tr>
<td>Age at inclusion (years) [median (IQR)]</td>
<td>54 (41-65)</td>
<td>48 (53-56)</td>
</tr>
<tr>
<td>Former smoking [n (%)]</td>
<td>18 (23)</td>
<td>20 (24)</td>
</tr>
<tr>
<td>Appendectomy [n (%)]</td>
<td>12 (16)</td>
<td>13 (16)</td>
</tr>
<tr>
<td>First-degree relative with auto-immune liver disease [n (%)]</td>
<td>3 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Positive Chlamydia IgG titer [n (%)]</td>
<td>30 (39)</td>
<td>22 (27)</td>
</tr>
</tbody>
</table>

PSC = primary sclerosing cholangitis; IBD = inflammatory bowel disease; OR = odds ratio; CI = confidence interval.

### Table 5.4 Univariable regression analysis Reovirus type 3

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>N</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>15 (60)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Age at time of inclusion (years) [median (IQR)]</td>
<td>47 (35-57)</td>
<td>57 (43-67)</td>
</tr>
<tr>
<td>Positive titer Reovirus type 3 neutralizing antibodies [n (%)]</td>
<td>9 (36)</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

PSC = primary sclerosing cholangitis; HC = healthy controls; IBD = inflammatory bowel disease; OR = odds ratio; CI = confidence interval.

### Geographical distribution

Of the total of 697 PSC patients, 218 PSC patients were diagnosed between 2000 and 2008 while living in the geographically defined area. When combining municipalities according to degree of urbanization, the observed number of PSC patients was not significantly different from the expected number of PSC patients (table 5.5). Incidence figures were not associated with degree of urbanization (p=0.97).
Chapter 5  Risk factors for PSC

Table 5.5 Geographical distribution

<table>
<thead>
<tr>
<th>Degree of urbanization (residences per km²)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed PSC cases</td>
<td>25</td>
<td>61</td>
<td>49</td>
<td>43</td>
<td>18</td>
</tr>
<tr>
<td>Expected PSC cases</td>
<td>30.8</td>
<td>52.9</td>
<td>48.5</td>
<td>44.5</td>
<td>19.2</td>
</tr>
<tr>
<td>SIR (95% CI)</td>
<td>0.81(0.53-1.18)</td>
<td>1.15(0.88-1.47)</td>
<td>1.01(0.75-1.33)</td>
<td>0.97(0.70-1.32)</td>
<td>0.94 (0.55-1.45)</td>
</tr>
</tbody>
</table>

PSC = primary sclerosing cholangitis; SIR = standardized incidence ratio; CI = confidence interval.

DISCUSSION

This is the largest case-control study showing that non-smoking behaviour is an independent risk factor for developing PSC. The negative association between smoking and PSC in the present study is in agreement with previous studies showing odds ratios of having PSC among smokers ranging from 0.33-0.37.21-23,22 The underlying mechanisms through which smoking affects the pathogenesis of PSC or IBD are largely unknown. Tobacco contains more than 4000 chemicals, of which nicotine is studied the most.23 Nicotine activates nicotinic acetylcholine receptors in the central nervous system, autonomic ganglia and neuromuscular junctions, but also on immune cells and in the mucosa and lymphoid tissue of small and large bowel.23 Van der Zanden et al. showed that repeated exposure to nicotine or tobacco smoking elevates α7 nicotinic acetylcholine receptor expression on human monocytes which may lead to sensitization of the ‘cholinergic anti-inflammatory pathway’.24 Placebo-controlled trials showed that addition of transdermal nicotine to maintenance therapy improves symptoms in UC patients,25,26 however transdermal or oral nicotine therapy had no short-term beneficial effect on symptoms or serum liver tests in PSC patients.27,28 More recently, nicotine has been shown to elevate mRNA expression of the pro-fibrogenic factors collagen 1-α2 and TGF-β (transforming growth factor beta) and induces human hepatic stellate cell proliferation.29 The effect of smoking on the progression of PSC has never been studied, yet in patients with primary biliary cirrhosis smoking has been associated with advanced histological disease at presentation.40 These findings may suggest that smoking is associated with a lower risk of developing PSC, but once liver fibrosis is present smoking might accelerate disease progression.

Although several authors have shown a protective effect of appendectomy on developing UC, in accordance with the present study a meta-analysis combining 342 PSC patients from four studies showed no association with PSC.21,23,24 In the present study we observed more appendectomies in PSC-UC patients and HC than in UC controls. These findings confirm the protective effect of appendectomy in UC. In the PSC-UC group the vast majority of appendectomies took place prior to UC diagnosis. These findings suggest that contrary to what has been observed in UC patients, appendectomy does not protect against developing UC in PSC patients. Perhaps this may be explained by a distinct genotype and IBD phenotype of PSC-UC patients.41 Possible immune-modulating effects of the appendix have been the focus of research for many years. A large study from Sweden performed between 1964 and 1993 including 212,963 patients showed that patients who underwent an appendectomy for an appendicitis or mesenteric lymphadenitis but not for non-specific abdominal pain before the age of 20 had a low risk of UC.42 These findings suggest that the inflammation rather than the absence of the appendix has a protective effect. Although the human biological pathways involved in the protective effect of appendicitis or complete removal of the appendix are just starting to be unravelled, an appendicitis mouse model showed down-regulation of 14 genes including IBD-associated genes after appendectomy.42

Bergquist et al. showed in a large Swedish study that first-degree family members of PSC patients have a 3.8-fold increased risk of PSC and a 3.3-fold increased risk of developing UC.43 A disadvantage of the present study is that relatives and friends accompanying PSC and UC in accordance with the present study a meta-analysis combining 342 PSC patients from four studies showed no association with PSC.21,23,24 In the present study we observed more appendectomies in PSC-UC patients and HC than in UC controls. These findings confirm the protective effect of appendectomy in UC. In the PSC-UC group the vast majority of appendectomies took place prior to UC diagnosis. These findings suggest that contrary to what has been observed in UC patients, appendectomy does not protect against developing UC in PSC patients. Perhaps this may be explained by a distinct genotype and IBD phenotype of PSC-UC patients.41 Possible immune-modulating effects of the appendix have been the focus of research for many years. A large study from Sweden performed between 1964 and 1993 including 212,963 patients showed that patients who underwent an appendectomy for an appendicitis or mesenteric lymphadenitis but not for non-specific abdominal pain before the age of 20 had a low risk of UC.42 These findings suggest that the inflammation rather than the absence of the appendix has a protective effect. Although the human biological pathways involved in the protective effect of appendicitis or complete removal of the appendix are just starting to be unravelled, an appendicitis mouse model showed down-regulation of 14 genes including IBD-associated genes after appendectomy.42

Lettle is known about the role of micro-organisms in the development of PSC. In 2002 Ponsioen et al. performed a serological screening for antibodies against 22 viruses, Chlamydia spp. and mycoplasma pneumoniae, showing a markedly elevated seroprevalence of Chlamydia antibodies in 41 PSC patients compared with IBD and healthy controls.27 In the present study we showed a similar elevated seroprevalence in PSC patients, however in multivariable analysis this association between a positive Chlamydia spp. IgA and IgG titer and PSC was not maintained. Reovirus type 3 antibodies have been associated with PSC once almost 30 years ago.26 The reason to study Reovirus type 3 infection in PSC is that it has been associated with neonatal biliary atresia, a progressive inflammatory obliteration of the extra- and intrahepatic bile ducts.43 However, a recent study by Rauschenfels et al. showed that hepatotropic viruses such as Reovirus type 3 are only present in a minority of biliary atresia patients and are unlikely to play a major role in the initiation or progression of biliary atresia.44 In the present study we found no association between presence of neutralizing antibodies against Reovirus type 3 and risk of developing PSC.

The even geographical distribution of PSC patients at time of diagnosis between cities and villages suggests that residential environment does not play a role in the etiopathogenesis
of PSC. In agreement with our findings, Ala et al. found no association between standardized prevalence ratios of PSC patients listed for liver transplantation in New York City and proximity to super fund toxic waste sites.45

This study has several limitations. The quality of a case-control study is very much dependent on the selection of controls. On average, three percent of all IBD patients develop concomitant PSC; therefore some IBD controls may develop PSC in the future, which should disqualify them as proper controls. Due to a distinct male/female ratio and age distribution at PSC diagnosis, sex and age matching was not possible in this case-control study, though by selecting controls randomly, comparison between groups was justified. Several confounding factors may play a role when studying smoking behaviour. Suffering from a life threatening chronic illness such as PSC may influence tobacco use. Notably, controls were recruited from outpatient clinics, which could have influenced the number of smokers. However, 27% of the general population (> 14 years of age) in the Netherlands was smoking in 2010, implying that the percentage of current smokers in the patient and control groups did not exceed the national average.46 Unfortunately, we did not register whether patients underwent an appendectomy for appendicitis, mesenteric lymphadenitis or for non-specific abdominal pain. No analysis on the occurrence of appendectomy in the PSC-CD vs CD subgroup was performed. The high frequency of ileocecal resection in CD patients introduces an important bias and hampers fair comparison of appendectomy frequency in this subgroup. When comparing total number of appendectomies, IBD controls and healthy controls have had more time at risk for developing appendicitis than PSC patients because appendectomies performed after PSC diagnosis were censored in the risk factor analysis. In conclusion, smoking is associated with a lower risk of developing PSC, independent of the protective effect in UC. Previous appendectomy for appendicitis, mesenteric lymphadenitis or for non-specific abdominal pain. No analysis on the occurrence of appendectomy in the PSC-CD vs CD subgroup was performed. The high frequency of ileocecal resection in CD patients introduces an important bias and hampers fair comparison of appendectomy frequency in this subgroup. When comparing total number of appendectomies, IBD controls and healthy controls have had more time at risk for developing appendicitis than PSC patients because appendectomies performed after PSC diagnosis were censored in the risk factor analysis. In conclusion, smoking is associated with a lower risk of developing PSC, independent of the protective effect in UC. Previous appendectomy is associated with decreased risk of UC, but not of PSC. The presence of antibodies against Chlamydia and Reovirus type 3 are not associated with PSC and degree of urbanization does not influence PSC incidence.

REFERENCES

Chapter 5

Risk factors for primary sclerosing cholangitis (PSC) include:

1. **Infectious agents**: Reovirus type 3 has been associated with PBC and PSC. A case-control study found a higher prevalence of reovirus 3 infection in patients with PBC compared to controls.

2. **Cigarette smoking**: Smoking is associated with nonsmoking behavior.

3. **Coffee consumption**: Coffee consumption is a risk factor for PSC, according to a case-control study in The Netherlands. A central role for coffee was also found in Southern Italy.

4. **Appendectomy**: A meta-analysis showed that appendectomy is associated with a reduced risk of inflammatory bowel disease.

5. **Nicotine**: Nicotine and acetylcholine receptor expression and susceptibility to cholinergic immunomodulation in human monocytes of smoking individuals. Nicotine induces fibrogenic changes in human liver. Nicotinic acetylcholine receptors expressed on hepatic stellate cells induce fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

6. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.


8. **Reovirus type 3**: Infection with reovirus type 3 has been linked to PBC and PSC.

9. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

10. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.


12. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

13. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.


15. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

16. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.

17. **Environmental factors**: Protective pathways against colitis mediated by appendicitis and appendectomy. A randomized double-blind placebo-controlled cross-over study.

18. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

19. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.


21. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

22. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.


24. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

25. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.


27. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

28. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.

29. **Environmental factors**: Protective pathways against colitis mediated by appendicitis and appendectomy. A randomized double-blind placebo-controlled cross-over study.

30. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

31. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.

32. **Environmental factors**: Protective pathways against colitis mediated by appendicitis and appendectomy. A randomized double-blind placebo-controlled cross-over study.

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45. **Nicotine**: Nicotine induces fibrogenic changes in human liver via nicotinic acetylcholine receptors expressed on hepatic stellate cells.

46. **Transdermal nicotine**: For the management of cholestatic liver diseases, transdermal nicotine was found to be beneficial in a placebo-controlled cross-over study.
Serum IgG4 and IgG1 for distinguishing IgG4-associated cholangitis from primary sclerosing cholangitis


* These authors contributed equally to this work
ABSTRACT

— Background
The recent addition of IgG4-associated cholangitis (IAC), also called IgG4-related sclerosing cholangitis (IRSC) to the spectrum of chronic cholangiopathies has created the clinical need for reliable methods to discriminate between IAC and the more common cholestatic entities primary (PSC) and secondary (SSC) sclerosing cholangitis. The current AASLD practice guidelines for PSC advise the measurement of sIgG4 in PSC patients, but interpretation of elevated sIgG4 levels remains unclear. We aimed to provide an algorithm to distinguish IAC from PSC using sIgG analyses.

— Methods
We measured total IgG and IgG subclasses in serum samples of IAC (n=73) and PSC (n=310) patients, as well as in serum samples of disease controls (primary biliary cirrhosis; n=22).

— Results
sIgG4 levels were elevated above the upper limit of normal (ULN=>1.4 g/L) in 45 PSC patients (15%, 95% CI 11-19). The highest specificity and positive predictive value (100%) for IAC were reached when applying the 4x ULN (sIgG4 >5.6 g/L) cut-off with a sensitivity of 42% (95% CI 31-55). However, in patients with a sIgG4 between 1x and 2x ULN (n=38/45) the PPV of sIgG4 for IAC is only 28%. In this subgroup, the sIgG4/sIgG1 ratio cut-off 0.24 yielded a sensitivity of 80% (95% CI 51-95), a specificity of 74% (95% CI 57-86), a PPV of 55% (95% CI 33-75) and a NPV of 90% (95% CI 73-97).

— Conclusion
Elevated sIgG4 (>1.4 g/L) occurred in 15% of patients with PSC. In patients with a sIgG4 >1.4 and <2.8 g/L, incorporating the IgG4/IgG1 ratio with a cut-off at 0.24 in the diagnostic algorithm significantly improved PPV and specificity. We propose a new diagnostic algorithm based on IgG4/IgG1 ratio that may be used in clinical practice to distinguish PSC from IAC.

INTRODUCTION
Primary sclerosing cholangitis (PSC) represents the most common chronic immune-mediated cholangiopathy among men, but is still rare given its prevalence ranging from 0 to 16.2 per 100,000 inhabitants. The differentiation of PSC from other chronic cholangiopathies can be extremely challenging. However, a correct diagnosis of PSC is crucial to optimize the surveillance of disease progression, as the chronic inflammation of the intra- and extrahepatic bile ducts may ultimately lead to cirrhosis and liver failure with median survival rates until death or liver transplantation from 12 to 21 years. PSC is more common in men than in women (2:1) and can occur at any age with a peak incidence around 40. PSC is strongly associated with inflammatory bowel diseases (IBD), often classified as ulcerative colitis, and patients have a poor prognosis due to liver failure and an increased risk for developing colorectal and biliary malignancies. The only known curative therapy available to date is orthotopic liver transplantation, although the use of ursodeoxycholic acid (UDCA) may improve surrogate markers of disease progression particularly in early stage patients and is still widely prescribed at moderate doses in non-fibrotic patients.

Discerning PSC from other chronic cholangiopathies has in recent years become even more challenging, with the establishment of a new disease entity IgG4-associated cholangitis (IAC), or IgG4 related sclerosing cholangitis (IRSC), with which PSC shares male predominance, a cholestatic serum enzyme pattern and cholangiographic features with intra- and/or extrahepatic bile duct strictures. IAC represents the biliary manifestation of a fully separate systemic disease entity: IgG4-related disease (IgG4-RD). IgG4-RD includes various organ manifestations among which pancreas and biliary tree appear most frequently affected. IgG4-RD is often associated with elevated IgG4 serum levels and is characterized by IgG4 positive plasmacellular tissue infiltrates. In striking contrast to PSC, IAC fully responds to corticosteroid treatment when diagnosed in time.

IgG4 is one of the four known subtypes of IgG molecules. Apart from being the least abundant IgG in healthy people (typically forming <5% of total serum IgG), IgG4 has unique biochemical properties, of which the ability to exchange its half molecules, thus yielding antibodies with dual antigen affinities, is the most intriguing. Unusually high IgG4 serum levels were first reported in patients with autoimmune pancreatitis (AIP) and have since then been associated also with other organ manifestations of IgG4-RD including IAC. A gold standard for diagnosing AIP and IAC is still lacking. Therefore, use of one of the currently accepted sets of diagnostic criteria has become common practice, of which the HISORt criteria for diagnosing AIP arguably are the most widely applied, and which were adapted for use in suspected IAC patients.
As IgG4-RD can be adequately controlled in the vast majority of cases by immunosuppressive medication, it is crucial to discriminate IAC cases from PSC patients. In the past, IAC becomes symptomatic, on average, in men of an older age (60-80 years). Upon careful history taking, IAC patients often have other organ manifestations of IgG4-RD, when compared to those with PSC. An elevated IgG4 serum level is still the pivotal finding that leads to the diagnosis, and elevated IgG4 levels are thus firmly anchored in the current diagnostic criteria of IAC. The AASLD practice guideline on the diagnosis and management of PSC published recently suggested measuring sIgG4 in all patients with possible PSC, to exclude IAC. However, elevated levels of sIgG4 have also been shown in 9-27% of clear-cut PSC patients. Therefore, it is currently unclear how to interpret an elevated sIgG4 level in a patient with alleged PSC. Furthermore, no data exists currently regarding the levels of other IgG subclasses in these patients. Given that specific IgG4-positive plasma/B-cell clones seem to play a central role in the pathogenesis of IAC, we hypothesised that IgG4 may be specifically increased in IAC whereas a non-specific increase in IgG of different IgG subclasses could be expected in other immune-mediated disorders such as PSC. Therefore, in search of a reliable algorithm we determined total IgG and IgG subclasses in serum samples of two large independent PSC and IAC cohorts as well as in serum samples of a disease control group with primary biliary cirrhosis (PBC).

PATIENTS AND METHODS

Study subjects

The Dutch (NL) cohort consisted of 132 PSC and 27 IAC patients. In addition, 22 PBC patients were included as liver disease controls. PSC and PBC patients were randomly selected from a large population-based study in the Netherlands (Epi PSC PBC study) containing 695 PSC patients and 1035 PBC cases included between March 2008 and December 2011. Between August 2004 and July 2012 IAC patients were referred to two large tertiary referral centres in the Netherlands: the Academic Medical Center in Amsterdam and the Erasmus University Medical Center in Rotterdam. The United Kingdom (UK) cohort consisted of 178 PSC and 46 IAC patients who were enrolled in a prospective database in the Oxford Radcliffe Hospitals from August 2010 onwards. The PSC patients were selected from a larger database of 346 patients attending the John Radcliffe Hospital in Oxford from between 2001 and 2012. PSC patients from the UK cohort were entered consecutively into a prospective PSC database at diagnosis or on referral to the centre. Patients were included in this study if full clinical details, biliary imaging and histology (where available) were reviewed and they had been followed-up in the hepatology clinic within the last 3 years. Serum samples were collected from 2005 onwards.

All medical records were thoroughly reviewed on site for confirmation of diagnosis and data retrieval. Patients with a PSC- or PBC-autoimmune hepatitis overlap syndrome and patients using corticosteroids and/or thiopurines during 6 months prior to serum sample collection were excluded. The study was approved by the Central Committee for Research Ethics in Utrecht and all participating local ethics committees in the Netherlands (www.trialregister.nl: NTR2813) and by the Oxford Research Ethics Committee (RECA 10/H0604/51) funded by a Wellcome Trust Fellowship grant [095160/Z/10/Z].

Case ascertainment

The diagnosis of PSC was based on: 1) clinical presentation i.e. pruritus, pain in the right upper abdominal quadrant, fatigue, weight loss, or episodes of fever and/or; 2) elevated alkaline phosphatase and gamma-glutamyltransferase, that was otherwise unexplained; 3) presence of characteristic bile duct changes with multifocal strictures and segmental dilatations on endoscopic retrograde cholangiography (ERC) or magnetic resonance cholangiography (MRC); 4) liver histology and 5) no evidence for secondary sclerosing cholangitis. Criteria 2 and 3 were considered mandatory for PSC, criteria 4 confirmed the diagnosis where available. No PSC patient was allowed to have pancreatic imaging suggestive of AIP or other organ involvement. The HISORt criteria (histology, imaging, serology, other organ involvement, and response to therapy) shown in figure 6.1 were applied for diagnosing IAC. The diagnosis of PBC was based on a combination of: 1) clinical presentation and/or; 2) elevation of alkaline phosphatase of liver origin for at least 6 months; 3) presence of anti-mitochondrial antibodies (≥1:40) in serum 4); and histological features of florid bile duct lesions. Criteria 2 and 3 were considered mandatory for PBC, criteria 4 confirmed the diagnosis where available.

Serology

Serum total IgG and subclasses IgG1, IgG2, IgG3, and IgG4 were measured using automated nephelometry (BN ProsPec Siemens in the Netherlands and BNII Siemens in Oxford). Samples were monitored for antigen excess by two separate strategies. Firstly IgG4 levels plus IgG1 levels for each patient were correlated to total IgG levels to check for >15% discordance, this gives an indication of antigen excess. Samples from patients with a normal IgG4 level were checked at multiple dilutions to check for non-linearity and probable antigen excess.

Statistical analysis

The Mann–Whitney U-test was performed for comparing continuous data without a normal distribution and t-tests were used to compare normally distributed continuous data. The chi-square test or Fisher’s exact test were used for comparing categorical data. The one-way ANOVA test and Kruskal-Wallis test were used for comparing continuous data between three

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groups. Receiver operator characteristic (ROC) curves were plotted to determine the optimal cut-off values for sIgG4 and for subclass ratio levels for distinguishing IAC from PSC. The optimal cut-off value was defined as the cut-off corresponding to the point on the ROC curve closest to the sens=1 spec=1 optimum. The diagnostic algorithms were compared using the McNemar test with regard to sensitivities and specificities and with the generalized score statistic as proposed by Leisenring with regard to PPV and NPV. Statistical analyses were performed using SPSS v. 19·0 software (Chicago, IL) and R (package DTComPair).

**RESULTS**

Elevated serum IgG4 (>1.4 g/L) occurs in 15% of PSC patients. In total, serum IgG and IgG subclasses were measured in 310 PSC, 73 IAC, and 22 PBC patients (demographics are shown in table 6.1). PSC patients were diagnosed at a mean age of 44.0 (SD 16.2), and IAC patients at a mean age of 62.5 years (SD 14.1) (p<0.001, t-test). Elevated sIgG4 levels (>1.4 g/L) were observed in 45 PSC patients (15%, 95% CI 11-19) (fig. 6.2). Seven (2%) had a sIgG4 greater than 2x ULN. None of the PSC patients had a sIgG4 greater than 4x ULN. Notably, 7 (10%) IAC patients had a sIgG4 < 1.4 g/L.

By: S. Ghazale, J. Alderlieste, and M. Maillet de Buy Wenniger.

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**Table 6.1** Demographics and serum total IgG and IgG subclasses of PSC and IAC patients and PBC controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PSC</th>
<th>IAC</th>
<th>p-value</th>
<th>PBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>310</td>
<td>73</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Male (n,%)</td>
<td>170 (55)</td>
<td>62 (85)</td>
<td>&lt;0.001</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Age at diagnosis (years) [mean (SD)]</td>
<td>44.0 (16.2)</td>
<td>62.5 (14.1)</td>
<td>&lt;0.001</td>
<td>49.5 (11.5)</td>
</tr>
<tr>
<td>Age at blood sampling (years) [mean (SD)]</td>
<td>51.2 (16.5)</td>
<td>64.1 (12.9)</td>
<td>&lt;0.001</td>
<td>60.0 (9.5)</td>
</tr>
<tr>
<td>IBD (n,%)</td>
<td>180 (58)</td>
<td>6 (8)</td>
<td>&lt;0.001</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total IgG (g/L) [mean (SD)]</td>
<td>13.5 (4.1)</td>
<td>17.0 (8.3)</td>
<td>0.01</td>
<td>13.5 (4.7)</td>
</tr>
<tr>
<td>IgG1 (g/L) [mean (SD)]</td>
<td>9.1 (3.5)</td>
<td>9.6 (5.0)</td>
<td>0.490</td>
<td>9.1 (3.2)</td>
</tr>
<tr>
<td>IgG2 (g/L) [mean (SD)]</td>
<td>3.5 (1.6)</td>
<td>4.5 (2.1)*</td>
<td>&lt;0.001</td>
<td>4.0 (1.9)</td>
</tr>
<tr>
<td>IgG3 (g/L) [median (IQR)]</td>
<td>0.4 (0.3-0.6)</td>
<td>0.4 (0.3-0.7)</td>
<td>0.288</td>
<td>1.2 (0.7-1.6)</td>
</tr>
<tr>
<td>IgG4 (g/L) [median (IQR)]</td>
<td>0.5 (0.3-1.0)</td>
<td>4.6 (2.2-11.1)</td>
<td>&lt;0.001</td>
<td>0.3 (0.2-0.5)</td>
</tr>
<tr>
<td>IgG4 &gt; 1.40 g/L (n,%)</td>
<td>45 (15)</td>
<td>66 (90)</td>
<td>&lt;0.001</td>
<td>0 (0)</td>
</tr>
<tr>
<td>IgG4 &gt; 2.80 g/L (n,%)</td>
<td>7 (2)</td>
<td>51 (70)</td>
<td>&lt;0.001</td>
<td>0 (0)</td>
</tr>
<tr>
<td>IgG4 &gt; 5.60 g/L (n,%)</td>
<td>0 (0)</td>
<td>31 (42)</td>
<td>&lt;0.001</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* based on 64 IAC patients. PSC = primary sclerosing cholangitis; IAC = IgG4-associated cholangitis; SD = standard deviation; IQR = inter quartile range.
When comparing PSC patients with an elevated sIgG4 to patients with normal sIgG4 levels, mean serum albumin levels were lower in patients with a sIgG4 >1.4 g/L (42 g/L (SD 5) vs. 44 g/L (SD 4), p=0.012, t-test). Median serum bilirubin levels (13 µmol/L (IQR 8-21) vs. 11 µmol/L (IQR 7-16), p=0.212, Mann–Whitney U-test) and alkaline phosphatase levels (323 U/L (IQR 175-578) vs. 290 U/L (IQR 175-485), p=0.414, Mann–Whitney U-test) were not significantly different. Mean age at PSC diagnosis of patients with an elevated sIgG4 did not differ from patients with a normal sIgG4 <1.4 g/L (45.3 years (SD 18.0) vs. 43.7 years (SD 15.8), p=0.551, t-test). The median time between PSC diagnosis and blood sampling was similar between groups (49 months (IQR 2-114) vs. 71 months (32-131), p=0.116, Mann–Whitney U-test.

Mean age at diagnosis was significantly different form IAC patients (45.3 years (SD 18.0) vs. 63.1 years (SD 13.5), p<0.001, t-test). All PSC patients with an elevated sIgG4 were scrutinised for signs of IAC. None of the PSC patients with an elevated sIgG4 had clinical signs or organ manifestations of IgG4-RD. Twenty-nine of 45 (64%) patients with PSC and an elevated sIgG4 had liver biopsies; of these 16 had tissue staining for IgG4 monoclonal antibody (table 6.2). Tissue IgG4 was >10/HPF in 3 of 16 liver biopsies (median 12, mean 20, range 12-35 IgG4/3 HPF). All 3 patients had a sIgG4 between 1.4 and 2.8g/l. Tissue IgG4 was <10/HPF in 13 liver biopsies, of which 9 had a serum IgG4 between 1.4 and 2.8g/l and 3 had a serum IgG4 >2.8g/l. None of the 16 biopsies showed histological characteristics of IAC. Conversely, IAC patients with a sIgG4 <1.4 g/L were reviewed for a possible diagnosis of PSC. None had concomitant IBD, 6 of 7 had pancreatic disease and responded to corticosteroid treatment, 1 of 7 with isolated IAC had classical histology and abundant IgG4 plasma cell staining in a liver resection specimen.

The majority of the IAC patients had pancreatic involvement. Eleven of 47 (23.4%) patients only in the Oxford cohort had IAC without pancreatic involvement; 8 of these 11 had other radiological and histologically-confirmed organ involvement including lung (2), renal (1), sialoadenitis (1) mesenteric fibrosclerosis (2), retroperitoneal fibrosis (1) and colonic (3) involvement. In the case of the colon, a lymphoplasmacytic infiltrate with abundant IgG4 cells (>50/HPF) was seen with no polypoid lesions, storiform fibrosis or phlebitis. Three of 47 (6.4%) patients had isolated IAC; two had resections to exclude cholangiocarcinoma with classical histology - lymphoplasmacytic infiltrate, storiform fibrosis and phlebitis - and IgG4-positive plasma cell counts of greater than 50/HPF, and one had an elevated serum IgG4 >4 times ULN, clinical, biochemical and radiological response with stricture resolution after 3 months of corticosteroids with no tissue diagnosis. Likewise, in the Dutch cohort the majority (88.9%) had pancreatic involvement at any point during follow-up, several cases also showed involvement of other organs such as the salivary glands. Only 3 of 27 (11%)
patients had isolated biliary IgG4-RD; none of these had other radiological or histologically-confirmed organ involvements, but all had elevated serum IgG4 and elevated numbers of IgG4-positive cells in their liver tissue, cholestasis and alterations on ERC or MRC suggestive of sclerosing cholangitis. All three showed a good clinical response to immunosuppressive treatment. When comparing the other sIgG subclass levels between PSC and IAC patients, IgG1 and IgG3 levels were similar and mean sIgG2 levels were higher in IAC patients than in PSC patients (4.5 vs. 3.5, p<0.001, t-test)(table 6.1).

The sIgG4 upper limit of normal cut-off is insufficient for distinguishing IAC from PSC. The upper limit of normal cut-off for sIgG4 (1.4 g/L) yields a sensitivity of 90% (95% CI 81-96) with a specificity of 85% (95% CI 81-89) for IAC (table 6.3). The positive predictive value (PPV) was only 59% (95% CI 50-69) whereas the negative predictive value (NPV) was 97% (95% CI 95-99). The vast majority (38/45) of PSC patients with a sIgG4 level >1.4 g/L fell in the >1.4 to <2.8 range, where the PPV for IAC was only 28%. Increasing the cut-off level to 2x ULN decreased the sensitivity of sIgG4 to 70% (95% CI 58-80), whereas the specificity and PPV increased to 98% (95% CI 95-99) and 88% (95% CI 76-95), respectively, and the NPV was only slightly reduced from 97% to 93% (95% CI 90-96). The highest specificity and positive predictive value (100%) for IAC were reached when applying the 4x ULN cut-off with a sensitivity of 42% (95% CI 31-55). In search of the optimal sIgG4 cut-off value for distinguishing IAC from PSC, we performed ROC analyses (fig. 6.3). We first determined the optimal cut-off value in the Dutch cohort, serving as a test cohort. Secondly, we tested the performance of this new cut-off value in the UK patients, serving as a validation cohort. The ROC curve of the test cohort showed that a sIgG4 cut-off at 2.5 g/L yielded the optimal combination of sensitivity and specificity (table 6.3). However, 7 (5%) PSC patients in the test cohort and 9 (5%) PSC patients in the validation cohort had a sIgG4 > 2.5 g/L and 3 (11%) IAC patients in the test cohort and 15 (33%) IAC patients in the validation cohort had a sIgG4 <2.5 g/L. These results illustrate the limited diagnostic value provided by moderately elevated sIgG4 when attempting to distinguish IAC from PSC.

Table 6.3 Performance of serum IgG4 in distinguishing IAC from PSC.

<table>
<thead>
<tr>
<th>IgG4 &gt;1.4 g/L</th>
<th>IgG4 &gt;2.8 g/L</th>
<th>IgG4 &gt;5.6 g/L</th>
<th>IgG4 &gt;2.5 g/L (NL cohort)</th>
<th>IgG4 &gt;2.5 g/L (Test Validation (UK) algorithm (figure 6.5))</th>
<th>New algorithm (figure 6.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity [% (95% CI)]</td>
<td>90 (81-96)</td>
<td>70 (58-80)</td>
<td>42 (31-55)</td>
<td>89 (70-97)</td>
<td>67 (52-80)</td>
</tr>
<tr>
<td>Specificity [% (95% CI)]</td>
<td>85 (81-89)</td>
<td>98 (95-99)</td>
<td>100 (99-100)</td>
<td>95 (89-98)</td>
<td>95 (90-98)</td>
</tr>
<tr>
<td>PPV [% (95% CI)]</td>
<td>59 (50-69)</td>
<td>88 (76-95)</td>
<td>100 (86-100)</td>
<td>77 (68-90)</td>
<td>78 (61-89)</td>
</tr>
<tr>
<td>NPV [% (95% CI)]</td>
<td>97 (95-99)</td>
<td>93 (90-96)</td>
<td>88 (84-91)</td>
<td>98 (93-99)</td>
<td>92 (87-95)</td>
</tr>
</tbody>
</table>

CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

Serum IgG4/IgG1 ratio is helpful in distinguishing IAC from PSC when sIgG4 is moderately elevated (>1.4 <2.8 g/L). Forty-five PSC patients (15%, 95% CI 11-19) had a sIgG4 above 1.4 g/L (fig. 6.2) and would erroneously be categorised as IAC patients. To determine if sIgG subclasses other than sIgG4 may help to reliably distinguish PSC from IAC in patients with an elevated sIgG4, we compared sIgG subclass levels between PSC patients with and without an elevated sIgG4,
and compared the subclass levels and slgG4 subclass ratios between PSC and IAC patients with a slgG4 >1.4 g/L. In PSC patients with a slgG4 >1.4 g/L mean slgG1 (ULN 11.4 g/L) and slgG2 (ULN 6.4 g/L) levels were higher than in PSC patients with a slgG4 <1.4 g/L (10.7 (SD 3.6) vs. 8.9 (SD 3.4), p=0.001 and 4.4 (SD 1.8) vs. 3.3 (SD 1.5), p<0.001, respectively). Median slgG3 (ULN 11 g/L) levels were not significantly different (0.5 (IQR 0.3-0.7) vs. 0.4 (IQR 0.3-0.6), p=0.103). When comparing PSC and IAC patients with a slgG4 >1.4 g/L, slgG1, slgG2 and slgG3 levels were not significantly different (data not shown). All slgG4 subclass ratios were higher in IAC patients than in PSC patients (table 6.4). To determine the most reliable subclass ratio for distinguishing IAC from PSC, we used ROC curves. The slgG4/slgG1 ratio showed the largest area under the curve (AUROC), and reached the optimal combination of sensitivity and specificity at 0.24 (table 6.4). In patients with a slgG4 >1.4 g/L the slgG4/slgG1 ratio cut-off value 0.24 yielded a sensitivity of 92% (95% CI 82-97), specificity of 64% (95% CI 49-78), a PPV of 79% (95% CI 68-87), and a NPV of 85% (95% CI 68-94). In patients with a slgG4 >2.8 g/L the slgG4/slgG1 ratio was not of additional value in distinguishing IAC from PSC. However, in patients with a moderately elevated slgG4 between 1x and 2x ULN the ratio cut-off 0.24 was of discriminating value with a specificity of 80% (95% CI 51-95), a specificity of 74% (95% CI 57-86), a PPV that improved from 28% to 55% (95% CI 33-75) and a NPV of 90% (95% CI 73-97) (fig. 6.4). A proposed algorithm for distinguishing IAC from PSC in all patients based on slgG4 and slgG1 levels is shown in figure 6.5.

<table>
<thead>
<tr>
<th>Subclass ratio</th>
<th>P value</th>
<th>AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>slgG4/slgG1 [median (IQR)]</td>
<td>0.21 (0.15-0.28)</td>
<td>0.61 (0.32-1.17)</td>
</tr>
<tr>
<td>slgG4/slgG2 [median (IQR)]</td>
<td>0.51 (0.41-0.51)</td>
<td>1.10 (0.68-2.13)</td>
</tr>
<tr>
<td>slgG4/slgG3 [median (IQR)]</td>
<td>4.46 (2.79-6.74)</td>
<td>10.14 (6.64-20.82)</td>
</tr>
</tbody>
</table>

PBC = primary sclerosing cholangitis; IAC = IgG4-associated cholangitis; AUROC = area under receiver operating curve; IQR = inter quartile range; CI = confidence interval.

Applying a diagnostic algorithm instead of the upper limit of normal as a diagnostic tool is helpful in distinguishing IAC from PSC. When using the upper limit of normal cut-off (1.4 g/L), 45 PSC patients (15%) would erroneously be diagnosed with IAC. When applying the algorithm shown in figure 6.5, 28 (62%) of these 45 PSC patients would not be classified as IAC on the basis of slgG4 profiles alone. In the remaining 17 (38%) patients the advice would be to look for characteristic histology of IAC. None of the PSC patients would be classified as having IAC instead of PSC solely based on slgG4 and corticosteroid treatment would only be started in patients with a slgG4 above 4x ULN or patients with histological signs of IgG4-RD. When considering all slgG4 values above 2x ULN or slgG4 values between 1x and 2x ULN together with a slgG4/slgG1 ratio above 0.24 as positive test results the proposed algorithm yielded a sensitivity of 86% (95% CI 76-93), a specificity of 95% (95% CI 91-97), a PPV of 79% (95% CI 68-87), and a NPV of 97% (95% CI 94-98) (table 6.3, last column). Compared to the sole use of the upper limit of normal (table 6.3, first column), this entailed a significant improvement in specificity and PPV (p<0.001 for both comparisons), with only minimal decline in sensitivity and NPV (p-values 0.25 and 0.19 respectively).

PBC patients have an increased serum IgG3.

Twenty-two PBC patients were included as cholestatic liver disease controls. Serum IgG1, IgG2, IgG4 and total IgG levels were within normal ranges. However, slgG3 was markedly elevated in PBC compared with PSC and IAC patients (median values 1.2, 0.4, and 0.4, respectively, p<0.001, Kruskal-Wallis test) (table 6.1). Eleven (50%) PBC patients had a slgG3 greater than the upper limit of normal.
DISCUSSION

The present study addresses the diagnostic dilemma of elevated serum IgG4 in differentiating patients with a chronic cholangiopathy otherwise compatible with PSC or IAC. Our data confirms that elevated serum IgG4 levels were found not only in IAC patients, but in a considerable fraction (15%) of a large cohort of PSC patients; that the PPV of a sIgG4 in the lower elevated range between 1.4 and 2.8 g/L for IAC was only 28%; and that, therefore, serum IgG4 was insufficient to discriminate between PSC and IAC. Our data for the first time shows that the serum IgG4/IgG1 ratio proved to be helpful in distinguishing IAC from PSC in patients with moderately elevated serum IgG4.

In line with our findings, previous case-series have shown elevated levels of IgG4 in 9% to 27% of PSC patients. However the number of included patients varied widely (34-285) and serum IgG1, IgG2, and IgG3 levels were never reported. In the current study, diagnostic accuracy provided by the sIgG4 only increased when increasing the sIgG4 cut-off value from 1.4 g/L to 2.8 g/L (2x ULN), or even to 5.6 g/L (4x ULN) confirming findings of others. Particularly when sIgG4 is moderately elevated between 1.4 and 2.8 g/L (12% of PSC patients) the risk of misclassification of PSC as IAC and inadvertent corticosteroid treatment was evident.

IgG4-related sclerosing cholangitis can be demonstrated on liver needle biopsy and bile duct biopsy. Affected bile ducts characteristically show diffuse thickening with transmural sclerosing inflammation composed of a dense lymphoplasmacytic infiltrate and storiform pattern of fibrosis. IgG4 immunostaining of infiltrating plasmacytes may be seen diffusely. Disease however may be patchy and can be missed on a regular biopsy specimen and histology should never be considered in isolation. The absolute IgG4 plasma count in a specimen can not be used in isolation and must form part of an assessment considering the clinical picture, histological morphology and the addition of an immunohistochemical IgG4 to IgG ratio (suggested at >40% to define IgG4-RD) as suggested by the consensus statement on the pathology of IgG4-RD. We feel that liver biopsy combined with cross sectional imaging for other organ manifestations, particularly pancreatic disease, is important diagnostically in a subgroup with PSC and elevated IgG4, particularly as they seem to constitute a high risk group. Histology is a crucial component to making an accurate diagnosis of IgG4-RD. Furthermore, whilst a biopsy carries a risk of complications in 5:1000 people, steroids can also cause many serious adverse effects. Therefore we feel that in patients with sclerosing cholangitis with equivocal results upon IgG spectrum measurement according to the proposed algorithm, liver histology should precede a trial of steroids.
In accordance with previous studies, 23% of PSC patients in our study had hypergammaglobulinemia.\textsuperscript{23} Hypergammaglobulinemia may include elevation of different IgG subtypes and may mirror continuous non-specific activation of the immune system. Whether elevated sIgG4 levels in this context are a cause or consequence of the severity of PSC remains elusive.

IAC patients showed on average an isolated elevation of IgG4, whereas, in PSC patients with an elevated IgG4, IgG1 was generally elevated as well, resulting in a lower IgG4/IgG1 ratio in PSC patients. Elevated sIgG4 levels in PSC have been associated with cirrhosis and a more severe disease course,\textsuperscript{19,21} but contamination of some PSC cohorts with undiagnosed IAC patients cannot be excluded – in one study several of the PSC patients with elevated IgG4 had a clinical profile suggestive of IgG4-RD, such as pancreatic involvement.\textsuperscript{21} Notably, cholangiocarcinoma is associated with elevated sIgG4, especially in association with PSC, and patients can have elevated sIgG4 levels, typically between 1x and 4x ULN.\textsuperscript{28} Data analyzing other IgG subclasses in CCA are not available.

Our data can help the interpretation of elevated serum IgG4 levels in the discrimination between IAC and PSC, but serum IgG subclass analyses do not represent 100% sensitive and specific tests. Therefore, the discrimination between IAC and PSC relies on more than serological measurements. Evaluating other diagnostic criteria for PSC or IAC such as age and co-existing IBD was beyond the scope of our study. PSC patients were not fully randomly selected from the two PSC cohorts, so bias with regard to age or co-existing IBD could not be excluded, and therefore we were unable to perform logistic regression for these factors. Nevertheless, in the present study, IAC patients were on average almost 20 years older at time of diagnosis than PSC patients (IAC; 62.5 years (SD 14.1) vs. PSC; 44.0 years (SD 16.2)). Even PSC patients with a sIgG4 >2.8 g/L were on average 13 years younger at time of diagnosis than IAC patients (data not shown). Furthermore, most IAC patients may upon careful history taking show to have various IgG4-RD organ manifestations. In addition, histopathological examination may help to differentiate between IgG4-RD and PSC.\textsuperscript{9}

In the present study, we observed higher sIgG3 levels in PBC patients compared with IAC and PSC patients. Anti-mitochondrial antibodies (AMA) - the diagnostic hallmark of PBC - are largely of IgG origin.\textsuperscript{32} AMA are not restricted to one IgG subclass, but AMA specific IgG3 titers are higher than IgG1 or IgG2 and associated with a more severe disease course.\textsuperscript{21,24}

Compared with PSC patients, IAC patients had higher sIgG2 levels. We have no pathophysiological explanation for the difference in IgG2 levels between IAC and PSC. It appears possible that both IgG2 and IgG4 subtypes are induced in IAC upon longstanding antigen exposure. Notably, IgG2 and IgG4 are both induced in response to polysaccharide antigens in contrast to IgG1 and IgG3.\textsuperscript{25} Another explanation could be that the measurement method is liable to false detection of slightly higher levels of IgG2 due to the great similarity of the IgG2 and IgG4 molecules.

There are several limitations to our study. Serum IgG subclasses of PSC and PBC patients were measured in frozen samples collected during the disease course instead of in fresh samples at time of diagnosis, as in IAC patients. We have done our utmost to ascertain the diagnosis in each patient, however we did not perform additional immunohistochemistry in all PSC patients for detection of IgG4+ plasma cells in PSC patients with an elevated sIgG4. However, none of the PSC patients had clinical signs of IgG4-RD (besides sclerosing cholangitis), which keeps the risk low that we misdiagnosed a PSC patient. Furthermore, cholangiocarcinoma in the setting of PSC can induce elevated levels of IgG4 in up to 22% of patients.\textsuperscript{28} In the present study, four (1%) PSC patients in retrospect probably had an undiagnosed cholangiocarcinoma at time of blood sampling; however, none had an elevated sIgG4.

In summary, we report the first analysis of IgG subclasses in the largest PSC and IAC patient group to date, as well as in PBC controls. We confirm that sIgG4 is elevated in 15% of patients with an unchallenged PSC diagnosis. Our study demonstrates that serum IgG4 >1.4 g/L is not reliable enough to detect IAC in alleged PSC patients with a moderately elevated sIgG4 (>1.4 <2.8 g/L). In this subgroup, incorporating the IgG4/IgG1 ratio with a cut-off at 0.24 in the diagnostic algorithm significantly improves PPV and specificity and is therefore helpful in distinguishing IAC from PSC. External validation of our findings is now warranted.

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REFERENCES


Rising incidence and prevalence of primary biliary cirrhosis: a large population-based study

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Chapter 7
Rising incidence and prevalence of PBC: a large population-based study

ABSTRACT

— Background

Large population-based studies are much needed to accurately establish the epidemiology of primary biliary cirrhosis (PBC). We aimed to collect all PBC patients in a geographically defined area in order to evaluate the epidemiology of PBC and examine the possible association of PBC with smoking, age at menarche, age at first pregnancy, and number of pregnancies.

— Methods

All PBC patients between 2000 and 2008 were identified in a geographically defined area of the Netherlands, comprising 50% of the Dutch population. Four independent hospital databases were searched in 44 hospitals. Medical records were reviewed on site verifying diagnosis and for collection of clinical data. Age- and gender-matched controls were recruited from the outpatient clinics of four participating hospitals. Patients and controls were asked to fill out a questionnaire regarding family history, previous and current smoking behaviour, and fertility status.

— Results

Nine hundred and ninety-two PBC patients fulfilled all inclusion criteria, resulting in a mean incidence of 1.1 per 100,000; 0.3 in men and 1.9 in women. On January 1st 2008 the point prevalence was 13.2 per 100,000 inhabitants. Incidence and prevalence rates were increasing over time (p<0.001). No geographical differences in disease distribution were observed. Smoking behaviour, age at menarche, age at first pregnancy, gravidity and number of children were not significantly different between cases and controls.

— Conclusion

Incidence and prevalence rates of PBC are increasing over time. PBC was not found to be associated with smoking, age at menarche, age at first pregnancy, or number of pregnancies.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease of unknown origin. Nowadays, it is generally believed that PBC is an autoimmune disease triggered by environmental factors in genetically susceptible individuals. The genetic component of PBC is well-established. Still, the role of risk factors is less clear. Female predominance in PBC and autoimmune diseases in general has led to the hypothesis that reproductive factors may trigger disease. Menarche, age at first pregnancy, and number of pregnancies are established indicators of intrinsic sex hormone exposure and have been associated with the risk of breast carcinomas as well as CRC. However, reports on the role of estrogens or gravidity in promoting PBC are conflicting. Epidemiology can be a powerful tool to yield important clues as to the etiology of diseases as well as the burden to society, but in order to it is paramount that sufficiently large, population-based figures are collected. Reported incidence and prevalence figures show quite some variation ranging from 0.33 to 5.8 per 100,000 and 1.9 to 40.2 per 100,000 inhabitants, respectively. These differences may be subject to the applied search strategy, the population under investigation, especially when relatively small populations are studied, and the degree of scrutiny of case-finding and ascertainment.

Herein, we report the largest population-based cohort study to date and a case-control sub study in order to evaluate the epidemiology of PBC in a large geographically defined area and examine the possible association of PBC with smoking, age at menarche, age at first pregnancy, or number of pregnancies.

METHODS

Design
Population-based observational cohort study and a case-control sub study.

Study area, population and period
Between 2008 and 2011 all PBC cases in 44 hospitals from 2000 onwards were identified in a geographically defined area of 6 adjacent provinces comprising 50% of the Dutch population (fig. 7.1). This area consists of 169 municipalities subdivided in 5 categories by degree of urbanization: 1; > 2500; 2; 1500-2500, 3; 1000-1500, 4; 500-1000, 5; <500 residences per km². The study was approved by the Central Committee for Research Ethics in Utrecht and all 44 local ethics committees of the participating hospitals in the Netherlands. The study is registered under NTR2813 (www.trialregister.nl).
Case-finding was performed according to guidelines stated by Metcalf and James. Four independent hospital databases were searched: 1) PALGA, nation wide network and comprehensive registry of histo- and cytopathology in the Netherlands; 2) nation wide hospital billing system using codes 707, 954, and 943 (primary sclerosing cholangitis/primary biliary cirrhosis/autoimmune hepatitis); 3) local clinical chemical laboratory database query of all patients with a positive anti-mitochondrial antibody test result, and 4) personal registry of physicians. In addition, PBC-registries in the three liver transplant centers in the Netherlands were checked for missed referrals from the area of interest.

Case-ascertainment
All medical records were evaluated on site (by KB and CP) for ascertainment of PBC diagnosis. The diagnosis of PBC was based on a combination of: 1) clinical presentation and/or; 2) elevation of alkaline phosphatase (AP) of liver origin for at least 6 months, and; 3) presence of anti-mitochondrial antibodies (≥1:40) in serum, and; 4) histological features of florid bile duct lesions. Criteria 2 and 3 were diagnostic for PBC, criteria 4 confirmed the diagnosis. PBC-Autoimmune hepatitis overlap syndrome (PBC-AIH) was defined as a combination of PBC and: 1) serum alanine transaminase (ALT) >5x upper limit of normal (ULN), and; 2) serum IgG >2x ULN or a positive test for anti-smooth muscle antibodies (ASMA); and 3) a liver biopsy showing moderate or severe perportal or periseptal lymphocytic piecemeal necrosis.

Controls
Age- and gender-matched controls were recruited from the outpatient clinics of four participating hospitals, equally distributed throughout the study area (fig. 7.1). Subjects without liver disease or autoimmune disease were eligible for inclusion.

Data collection
At study inclusion the following data were collected: date of PBC diagnosis, zip code of residence at time of diagnosis. Patients and controls were asked to give informed consent and fill out a 10-item questionnaire regarding family history, previous and current smoking status, and fertility status. Year, age-, and sex-specific population estimates were retrieved from the Dutch Central Office for Statistics (Centraal Bureau voor de Statistiek, Den Haag, the Netherlands; http://statline.cbs.nl).

Statistical analysis
Prevalence and incidence rates were expressed per 100,000 inhabitants (≥ 20 years of age) per year. Cochrane-Armitage test for trend was used to test changes in prevalence and incidence. Standardized incidence ratios (SIRs) were calculated per municipality to check for spatial differences. SIR is the ratio of observed compared with expected number of cases. The One-Way ANOVA test was used to assess differences in incidence rates between municipalities subdivided according degree of urbanization. We examined reproductive factors among females only. Number of pregnancies, age at first pregnancy, and gravidity for cases and controls above 45 years of age at time of inclusion were compared, assuming that these women are no longer capable of reproduction. T-tests were used to assess differences in means. The chi-square test or Fisher’s exact test were used for categorical data. Statistical analyses were performed using SPSS version 19.0 software (Chicago, IL). P < 0.05 was considered statistically significant.
RESULTS

Study inclusion
The case-finding queries yielded 3090 unique cases. Of these, 1035 fulfilled the diagnostic criteria for PBC and were alive on January 1st 2000. Forty-three cases were living outside the study region, leaving 992 PBC patients eligible for inclusion in the study cohort (fig. 7.1). Reasons for study exclusion are depicted in figure 7.2. In total, 464 cases and 128 age- and gender-matched controls completed the questionnaire.

![Flowchart patient inclusion](image)

Patient characteristics
Among the 992 patients, 121 were male (12%), and the mean age at diagnosis was 61 (SD; 12) in men and 57 (SD; 13) in women (p<0.001). At time of inclusion, 85 (9%) patients fulfilled the criteria for autoimmune hepatitis overlap syndrome.

Incidence and prevalence
The total number of inhabitants (≥ 20 years of age) increased during the study period from 5,569,825 in 2000 to 5,855,630 in 2007. The mean annual incidence between 2000 and 2007 was 1.1 per 100,000; 0.3 in men and 1.9 in women (fig. 7.3). On January 1st 2008 the point prevalence was 13.2 per 100,000 inhabitants. The highest incidence and prevalence rates were observed in women between ages 70-79 (4.2 and 42.3, respectively). Incidence and prevalence rates increased over time (p<0.001) (fig. 7.4 and 7.5). Net growth was attributable to increase in incidence and not to a decrease in number of deaths (p=0.007, Cochrane-Armitage test for trend).

Geographical distribution
In order to check for spatial differences, standardized incidence ratios were calculated for all 169 municipalities in the study region. Geographical differences in incidence rates were observed for the municipalities Almere and Hoogeveen. In Almere, we observed a lower incidence rate than expected based on number of inhabitants (SIR 0.37; 95% CI 0.12-0.87). The incidence rate was significantly higher in Hoogeveen (SIR 2.33; 95% CI 1.12-4.28). The incidence rates coincided with a relatively young population in Almere and older population in Hoogeveen as shown in figure 7.6. Mean incidence rates did not differ significantly between municipalities subdivided according degree of urbanization (fig. 7.7).

Lifestyle, reproductive, and familial factors
Among cases and controls, 20% and 17% reported a past history of smoking, respectively (p=0.455). Current smoking was similar in both groups; 20% in cases and 19% in controls (p=0.778). No differences in age at menarche, age at first pregnancy, gravidity and number of children were observed among cases and controls (table 7.1).

DISCUSSION
We here present the largest population-based study of PBC. Increasing disease awareness, improved diagnostic tools, availability of an effective therapy, and digitalized patient registration likely contributed to the rising incidence and prevalence rates observed between 1969 and 1999. A large study from Finland showed increasing prevalence rates between 1988 and 1999. The increase in prevalence was due to an increasing incidence and improved survival. However, we observed an increase in incidence and prevalence rates from 2000-2008, independent of number of deaths, yet patient registration, diagnostic approach and therapy remained unchanged over these years. This suggests that there is a true increase in PBC occurrence, rather than increase in detection and reporting or improved survival.
Chapter 7

Rising incidence and prevalence of PBC: a large population-based study

Figure 7.3 Age- and gender-specific incidence PBC per 100,000 inhabitants per year.

Figure 7.4 Gender specific PBC incidence per 100,000 inhabitants per year. Total incidence rates increased over time (p<0.001, Cochrane-Armitage test for trend).

Figure 7.5 Gender specific point prevalence PBC per 100,000 inhabitants per year. Total prevalence rates increased over time (p<0.001, Cochrane-Armitage test for trend).

Figure 7.6 Age distribution in municipalities Almere and Hoogeveen compared to the average age distribution in the Netherlands.
Chapter 7 Rising incidence and prevalence of PBC: a large population-based study

Reported incidence and prevalence figures show quite some variation ranging from 0.33 to 5.8 per 100,000 and 1.91 to 40.2 per 100,000 inhabitants, respectively. Most epidemiological studies on PBC have been performed in specialized centers, prone to selection and referral bias. We observed similar incidence and prevalence figures as found in Sweden and Norway. However, these studies were performed between 1973 and 1995. Between 1988 and 1999 a large study was performed in Finland showing an incidence of 1.7 and prevalence of 18.0 per 100,000 inhabitants. Recently, a population-based study from Iceland reported an even higher crude incidence of 3.4 and a prevalence of 38.3 in a background population of 317,630 inhabitants. Case-finding and case-ascertainment strategies were comparable to our study, yet incidence and prevalence rates are three times higher. The populations of Finland and Iceland are quite homogeneous and almost entirely from Northern European ancestry, and so far, the highest incidence and prevalence rates have been found in Northern Europe and North America. These observations have led to the hypothesis that there is a north-south gradient, which may explain the strikingly high occurrence in Iceland. However, it is important to take into account that until 2005 no studies were performed outside the Western world. Compared to our study, the catchment areas in previous population-based studies were manifold smaller, rendering them more vulnerable to sampling error. Limitations of this study lie in its retrospective nature with its immanent risk of incomplete datasets. Although we have extensively searched all hospitals in the predefined geographic area and the potential tertiary referral hospitals, there is always a chance of missing some cases and therefore slightly underestimating incidence and prevalence. However, by combining several databases and visiting all hospitals in a geographically defined area we feel that the chance of missing data is limited.

The striking female predominance in PBC and autoimmune diseases in general has led to the hypothesis that reproductive factors may trigger disease. Menarche, age at first pregnancy, and number of pregnancies are established indicators of intrinsic sex hormone exposure and have been associated with the risk of breast carcinomas as well as CRC. PBC is not only a female predominant disease, but also tends to occur after the childbearing years. In the present study no association between reproductive factors and PBC was found.

Meta-analysis combining five large well-conducted case-control studies on environmental, familial, and medical factors showed that among others, a history of cigarette smoking was associated with PBC. On the contrary, in the largest conducted study by Gershwin et al. controls were more often current smokers. Several confounding factors may play a role when studying smoking behaviour. The authors recognize that an explanation for differences in smoking behaviour could be the dissimilarity in socioeconomic status between cases and controls.
controls. Recent studies show that a history of cigarette smoking is associated with the severity of liver fibrosis at diagnosis. This could indicate that smoking PBC patients have a more severe disease course and are more likely to have received medical attention, which may constitute a confounder. Moreover, none of the case-control studies were population-based with an inevitable risk of referral and selection bias. In our population-based study, neither a positive nor a negative association with past or current smoking behaviour was found. Notably, controls were recruited from a hospital population which could have influenced the number of smokers. However, 27% of the general population (> 14 years of age) in the Netherlands was smoking in 2010, implying that the percentage of current smokers in the patient and control group did not exceed the national average. The lower number of controls than cases is a limitation of our study. Still, by recruiting age- and gender matched controls from four different areas in the Netherlands, we have tried to compose a representative control group.

Besides infectious, lifestyle, and reproductive factors, several environmental triggers for PBC have been studied in the last thirty years. Two studies from the United Kingdom showed strong variations in geographical distribution of cases. However, no potential triggers could be identified. In New York, a significant association between a cluster of PBC patients and superfund toxic waste sites contaminated with volatile aromatic hydrocarbons and trichloroethylene was identified, supporting the hypothesis that environmental toxins play a role in development of PBC. We observed a lower PBC incidence in Almere and a higher incidence in Hoogeveen. However, these differences coincided with a relatively young population in Almere and older population in Hoogeveen compared to the average age distribution in the Netherlands. With a mean age at diagnosis of 61 years, differences in age distribution could explain the observed incidence rates. The geographical distribution of patients in our study suggests that there is no role for environmental toxin exposure in the etiopathogenesis of PBC. Our findings do not exclude an association with an equally distributed toxin, nor with exposure to a toxin long before a diagnosis is made when cases may have lived or worked elsewhere.

In conclusion, we report increasing incidence and prevalence rates of PBC in a large population-based cohort of 992 patients. This population-based study did not confirm smoking or sex hormone exposure as risk factors for PBC. Moreover, no geographical differences in disease distribution were observed.

REFERENCES


Increased cancer risk in a large population-based cohort of patients with primary biliary cirrhosis: Follow-up for up to 36 Years
ABSTRACT

— Background

The natural history of primary biliary cirrhosis (PBC) has so far mainly been studied in tertiary referral centers. The aim of the present investigation was to describe the natural history of PBC in a large population-based cohort in order to identify risk factors for development of malignancies and disease progression.

— Methods

Four independent hospital databases were searched in 44 hospitals in a geographically defined area, after which all medical records were evaluated on site. In addition, PBC registries in the three liver transplant centers were checked for missed referrals from the area of interest.

— Results

In total, 992 cases fulfilled the inclusion criteria. The median follow-up was 73 months (range 0-434). Mortality was similar to the age- and gender-matched population (SMR 1.1; 95% CI 0.9-1.4). Male gender, smoking, and elevated bilirubin, decreased albumin, and elevated AST at time of diagnosis, were associated with an increased risk for the combined endpoint PBC-related death or liver transplantation. In total, 133 (13%) patients developed one or more malignancies (SIR 1.5; 95% CI 1.1-1.9). There was a 9-fold increased risk of developing hepatobiliary malignancies (SIR 9.4; 95% CI 3.04-21.8), a 5-fold increased risk of developing urinary bladder cancer (SIR 5.0; 95% CI 1.6-11.6), and a 1.8-fold increased risk of developing breast cancer (SIR 1.8; 95% CI 1.08-2.81).

— Conclusion

PBC is associated with an increased risk of hepatobiliary, bladder and breast cancer. Still, survival - under treatment with UDCA - was comparable to the general population in this population-based study.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic disease of unknown etiology characterised by destruction of small and medium sized bile ducts. When medical therapy was not yet available for PBC, the estimated median survival from diagnosis until death was 8.4 years. Two major changes in the treatment of PBC were the introduction of liver transplantation as a standard treatment for liver diseases and the first clinical trials with ursodeoxycholic acid (UDCA) in 1982. Between 1968 and 1986 primary biliary cirrhosis was the primary indication for liver transplantation in Europe. However, the number of PBC patients listed for receiving a liver transplantation has declined significantly since 1988, together with widespread use of UDCA, a semi synthetic hydrophilic bile acid that has been shown to improve biochemical and histological markers, and subsequently to slow progression and improve survival. PBC has been associated with an increased risk of malignancies, mainly hepatocellular carcinoma (HCC). Several risk factors have been associated with HCC development in PBC including advanced histological stage, male gender, cigarette smoking, history of blood transfusion, absence of biochemical response to UDCA, and concomitant hepatitis B and C infection. However, reports on cancer risk and contributing factors are conflicting. These contradictory findings may partly be explained by a lack of large population-based cohort studies.

Several models predicting survival of PBC patients at time of diagnosis have been developed over the years. These models had disadvantages in that they required a liver biopsy, applied subjective parameters, and laboratory results without adjustment for differences in normal values between hospitals. More recent models evaluate the long-term prognosis of PBC patients under UDCA treatment in relation to their biochemical response to treatment after 1-2 years. However, these models do not predict survival at time of diagnosis, which is a disadvantage in counselling patients. Ter Borg et al. developed a simple and inexpensive method to predict survival based on serum bilirubin and albumin levels at time of diagnosis, known as the Rotterdam survival model. Here we describe the natural history of PBC in a large population-based cohort in order to identify risk factors for development of malignancies and disease progression, and to evaluate the prognostic value of the Rotterdam survival model.
PATIENTS AND METHODS

This study is part of the Epi PSC PBC project, a large population-based epidemiological study of PSC and PBC in the Netherlands. The case-finding and case-ascertainment methods have been described previously.21

Study population and period

All PBC patients were identified between February 2008 and December 2011 in 44 hospitals in a geographically defined area of 6 adjacent provinces comprising 50% of the Dutch population. The study was approved by the central Committee for Research Ethics in Utrecht and all 44 local ethics committees of the participating hospitals in the Netherlands and registered under NTR2813 (www.trialregister.nl).

Case-finding

Case-finding was performed according to guidelines stated by Metcalf and James.24 Four independent hospital databases were searched: 1) PALGA nationwide network and registry of histo- and cytopathology reports using diagnosis code liver*biopsy*primary biliary cirrhosis (M4559OT56000); 2) nation wide hospital billing system using codes 707, 954, and 943 (primary sclerosing cholangitis/primary biliary cirrhosis/autoimmune hepatitis); 3) central and local clinical chemical laboratory database query of all patients with a positive anti-mitochondrial antibody test result, and 4) personal registry of physicians. In addition, PBC-registries in the three liver transplant centers in the Netherlands were searched for missed referrals from the area of interest.

Case-ascertainment

All medical records were evaluated on site for ascertainment of PBC diagnosis. The diagnosis of PBC was based on a combination of: 1) clinical presentation and/or; 2) elevation of alkaline phosphatase (AP) of liver origin for at least 6 months; 3) presence of anti-mitochondrial antibodies (≥1:40) in serum; and 4) histological features of florid bile duct lesions. Criteria 2 and 3 were diagnostic for PBC, criteria 4 confirmed the diagnosis.26 PBC autoimmune hepatitis overlap syndrome (PBC-AIH) was defined as a combination of PBC and: 1) serum alanine transaminase (ALT) >5x upper limit of normal (ULN); 2) serum IgG >2x ULN or a positive test for anti-smooth muscle antibodies (ASMA); and 3) a liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis.27

Data collection

At study inclusion and during follow-up the following data were collected: date of PBC diagnosis, serum bilirubin, albumin and aspartate transaminase (AST) levels at time of diagnosis, date and type of malignancy, date and type of surgery and medication use. Patients were subdivided according to the Rotterdam survival model in three categories at time of diagnosis: 0) normal serum bilirubin (<17 µmol/L) and albumin (35-50 g/L) levels; 1) decreased albumin or increased bilirubin levels, and; 2) decreased albumin and increased bilirubin levels. Only patients who were not present in the initial cohort of ter Borg et al.22 were included in the validation analysis. All patients were asked to give informed consent and to fill out a questionnaire regarding onset of symptoms, previous and current smoking status, weight and length. After study inclusion, individual medication history from the local pharmacies and annual follow-up from the treating physician was obtained by written correspondence. End of follow up was defined as death, last visit to outpatient clinic or end of study (May 2012). PBC-related death was defined as death caused by liver failure, variceal bleeding or hepatobiliary malignancy. Date of death was retrieved from the national death registry. Mid-year, age-, and sex-specific population estimates were based on data from the Dutch Central Office for Statistics (http://statline.cbs.nl/statweb/). Data on the incidence of malignancies in the general population were retrieved from the Dutch Cancer Registry (www.cijfersoverkanker.nl).

Statistical analysis

Time of diagnosis was defined as the starting point of the disease for all analyses. Kaplan-Meier survival analysis was performed to estimate the cumulative survival. Estimated median survival times were calculated for the combined endpoint PBC-related death or liver transplantation. The overall difference in survival was investigated by the log-rank test. The standardized mortality ratio (SMR) and standardized incidence ratio (SIR) were calculated as the ratios of observed compared to expected number of deaths and malignancies in the study cohort. The expected number of patients was calculated based on the age- and gender specific mortality and malignancy rates in the general population. Cox-regression analysis was performed for univariate and multivariate analysis of risk factors for endpoints PBC-related death and liver transplantation. Potential risk factors for developing malignancies were assessed using multivariable logistic regression. Statistical analyses were performed using SPSS v.19.0 software (Chicago, IL). P < 0.05 was considered statistically significant.

RESULTS

Study inclusion

The case-finding queries yielded 3090 unique cases. Of these, 1035 cases fulfilled the diagnostic criteria for PBC and were alive on January 1st 2000. Forty-three cases were living outside the study region, leaving 992 PBC patients eligible for inclusion in the study.
cohort. Reasons for study exclusion are depicted in figure 7.2. Between February 2008 and December 2011, 657 patients were alive and deemed capable of answering a questionnaire. In total, 459 (70%) patients completed the questionnaire.

Patient characteristics
The median follow up from diagnosis until death or end of study was 73 months (range 0-434). Year of diagnosis ranged from 1973 until 2011. Among the 992 patients, 121 were male (12%), and the mean age at diagnosis was 61 (+/- SD: 12) in men and 57 (+/-SD: 13) in women (p<0.001). At time of inclusion, 85 (9%) patients fulfilled the criteria for autoimmune hepatitis overlap syndrome. In 865 patients, medication data could be retrieved. 801 (93%) of patients were treated with UDCA.

Natural history
Survival
During follow up, 17 (2%) patients underwent liver transplantation and 145 (15%) patients died. Causes of death are depicted in table 8.1. Overall, mortality was similar to the age- and gender-matched population (SMR 1.1; 95% CI 0.9-1.4). Survival until transplantation or PBC-related death was 100% after 1 year, 98% after 5 years, 95% after 10 years, and 91% after 20 years (fig. 8.1). For men, survival until liver transplantation or PBC-related death was significantly decreased compared with women (log-rank p<0.001) (fig. 8.2). Still, the overall mortality for men was comparable with the general male population (SMR 1.4; 95% CI 0.8-2.3).

Male gender, smoking (≥ 1 year past 20 years), and elevated bilirubin, decreased albumin, and elevated AST levels at time of diagnosis, were associated with an increased risk for the combined endpoint PBC-related death or liver transplantation in univariate analysis (table 8.2). Multivariate cox-regression analysis showed that male gender (HR 4.03; 95% CI 1.35-12.0), elevated bilirubin (HR 10.10; 95% CI 2.19-46.6) and decreased albumin levels (HR 5.68; 95% CI 1.85-17.4) at time of diagnosis were independent risk factors for the combined endpoint.

Rotterdam survival model
Serum bilirubin and albumin levels at diagnosis were available for 420 (42%) patients. When excluding patients present in the initial cohort of ter Borg et al. 371 (37%) patients were eligible for external validation of the Rotterdam survival model. Survival until liver transplantation or PBC-related death of patients with normal serum bilirubin and albumin levels at time of diagnosis was 100% after 1 year, 99% after 5 years, and 99% after 10 years; for patients with an abnormal serum bilirubin or albumin level it was 100%, 96%, and 81%, respectively; and for patients with abnormal bilirubin and albumin levels it was 93%, 66%, and 66%, respectively (fig. 8.3). Among patients with abnormal bilirubin and albumin levels,
Increased cancer risk in a large population-based cohort of patients with PBC

with the general population (SIR 9.4; 95% CI 3.0-21.8). The hepatobiliary malignancies consisted of 7 hepatocellular carcinomas (HCC) and one cholangiocarcinoma. Median age at HCC diagnosis was 67 years (range 59-82) and the median time between PBC diagnosis and HCC was 24 years (range 9-29). Gender distribution, smoking, and the prevalence of concomitant autoimmune hepatitis were similar in patients with and without HCC (table 8.4).

Table 8.2 Risk factors for PBC-related death or liver transplantation.

<table>
<thead>
<tr>
<th>Factor</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>3.280</td>
<td>1.653-6.507</td>
<td>0.001</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>1.007</td>
<td>0.982-1.033</td>
<td>0.601</td>
</tr>
<tr>
<td>Concurrence of AIH</td>
<td>1.580</td>
<td>0.622-4.017</td>
<td>0.337</td>
</tr>
<tr>
<td>Smoking ≥ 1 year past 20 years</td>
<td>2.949</td>
<td>1.088-7.996</td>
<td>0.034</td>
</tr>
<tr>
<td>Bilirubin &gt;16 µmol/L at diagnosis</td>
<td>17.685</td>
<td>5.035-62.110</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin &lt;35 g/L at diagnosis</td>
<td>8.649</td>
<td>3.274-22.848</td>
<td>0.001</td>
</tr>
<tr>
<td>AST &gt;39 U/L at diagnosis</td>
<td>4.338</td>
<td>1.278-14.728</td>
<td>0.019</td>
</tr>
<tr>
<td>UDCA</td>
<td>0.726</td>
<td>0.254-2.069</td>
<td>0.548</td>
</tr>
</tbody>
</table>

AIH: autoimmune hepatitis; AST: aspartate transaminase; UDCA: ursodeoxycholic acid; HR: hazard ratio; CI: confidence interval.

Table 8.3 Malignancies in 133 PBC patients

<table>
<thead>
<tr>
<th>n</th>
<th>SIR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>32</td>
<td>1.8</td>
</tr>
<tr>
<td>Colon</td>
<td>27</td>
<td>1.4</td>
</tr>
<tr>
<td>Gynaecological</td>
<td>15</td>
<td>0.6</td>
</tr>
<tr>
<td>Skin</td>
<td>13</td>
<td>NA</td>
</tr>
<tr>
<td>Hepatobiliary tract</td>
<td>8</td>
<td>9.4</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>8</td>
<td>NA</td>
</tr>
<tr>
<td>Prostate</td>
<td>6</td>
<td>2.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>6</td>
<td>2.0</td>
</tr>
<tr>
<td>Bladder</td>
<td>5</td>
<td>5.0</td>
</tr>
<tr>
<td>Gastric</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>Lung</td>
<td>4</td>
<td>NA</td>
</tr>
<tr>
<td>Leukemia</td>
<td>4</td>
<td>NA</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Larynx</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Unknown origin</td>
<td>2</td>
<td>NA</td>
</tr>
</tbody>
</table>

n: number; SIR: standardized incidence ratio; CI: confidence interval; NA: not applicable

Figure 8.2 Kaplan-Meier analysis of survival until liver transplantation or PBC-related death in male and female PBC patients (log-rank p<0.001).

Figure 8.3 Kaplan-Meier analysis of survival until liver transplantation or PBC-related death in PBC patients subdivided according to the Rotterdam survival model: 0) normal serum bilirubin and albumin (n=245); 1) increased serum bilirubin or decreased serum albumin (n=93); and 2) increased serum bilirubin and decreased serum albumin (n=33).
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Table 8.4 Risk factors for developing malignancies in PBC

<table>
<thead>
<tr>
<th></th>
<th>HCC</th>
<th>No HCC</th>
<th>OR (95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex (%)</td>
<td>1 (14)</td>
<td>120 (12)</td>
<td>1.71 (0.20-14.91)</td>
<td>0.626</td>
</tr>
<tr>
<td>AIH overlap (%)</td>
<td>1 (14)</td>
<td>84 (9)</td>
<td>1.57 (0.18-13.56)</td>
<td>0.684</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>1 (14)</td>
<td>294 (30)</td>
<td>0.30 (0.04-2.52)</td>
<td>0.267</td>
</tr>
<tr>
<td>Age at diagnosis [mean (SD)]</td>
<td>47 (13)</td>
<td>57 (13)</td>
<td>0.94 (0.89-1.00)</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Bladder cancer

<table>
<thead>
<tr>
<th></th>
<th>n=5</th>
<th>n=987</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex (%)</td>
<td>1 (20)</td>
<td>120 (12)</td>
<td>1.37 (0.14-13.20)</td>
</tr>
<tr>
<td>AIH overlap (%)</td>
<td>1 (20)</td>
<td>84 (9)</td>
<td>2.59 (0.28-24.35)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>2 (40)</td>
<td>293 (30)</td>
<td>1.80 (0.28-11.58)</td>
</tr>
<tr>
<td>Age at diagnosis [mean (SD)]</td>
<td>62 (16)</td>
<td>57 (13)</td>
<td>1.04 (0.96-1.11)</td>
</tr>
</tbody>
</table>

Female PBC patients only

<table>
<thead>
<tr>
<th></th>
<th>Breast cancer</th>
<th>No breast cancer</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIH overlap (%)</td>
<td>2 (6)</td>
<td>67 (8)</td>
<td>0.81 (0.19-3.49)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>11 (34)</td>
<td>243 (30)</td>
<td>1.50 (0.69-3.23)</td>
</tr>
<tr>
<td>Age at diagnosis [mean (SD)]</td>
<td>61 (13)</td>
<td>57 (13)</td>
<td>1.03 (0.99-1.06)</td>
</tr>
</tbody>
</table>

AIH: autoimmune hepatitis; HCC: hepatocellular carcinoma; n: number; UDCA: standard deviation; OR: odds ratio; CI: confidence interval.

Older age at diagnosis decreases the risk of HCC. All PBC-HCC patients received UDCA treatment prior to HCC diagnosis. However, 4 out of 7 HCC patients were diagnosed with PBC between 1979 and 1981, when UDCA was not yet administered. Five PBC patients developed bladder cancer; a 5-fold higher incidence rate than expected (SIR 5.0; 95% CI 1.6-11.6). Gender distribution, age at diagnosis, smoking and AIH overlap were similar in patients with and without bladder cancer (table 8.4). The risk of breast cancer was 1.8 fold higher in female PBC patients compared with the age-matched female population (SIR 1.8; 95% CI 1.08-2.81). The development of breast cancer was not associated with smoking, age at diagnosis, or AIH overlap syndrome (table 8.4).

DISCUSSION

This study is by far the largest population-based study of PBC, based on an extensive case-finding strategy and rigorous case ascertainment in a defined recruitment area. We report a markedly longer survival until transplantation or PBC-related death compared to previous studies. Most epidemiological studies on PBC have been performed in specialized centers, prone for selection and referral bias. In the present study, overall survival of PBC patients is comparable to the general population, however, male gender, smoking, and elevated serum liver tests at diagnosis are associated with an increased risk for the combined endpoint liver transplantation or PBC-related death. The present analysis confirms the prognostic value of the Rotterdam survival model, stratifying patients based on serum bilirubin and albumin levels at the time of diagnosis. Notably, 30% of patients with abnormal serum bilirubin and albumin levels at diagnosis had concomitant AIH. More recent studies showed that serum AP after one year of UDCA treatment appears to be a useful prognostic marker in early stage PBC. However, the limited number of included patients asks for validation in an independent cohort. Currently, we take part in a large international effort aimed at cross-validating these promising results.

The introduction of UDCA therapy in the late 80’s and 90’s altered the course of PBC. Mendes et al. observed a decline in mortality in women younger than 65 and an increase in age of death between 1980 and 1998, pointing towards an improvement in survival. UDCA therapy appears to be of most benefit when started in the early histological stages of the disease. In our cohort, 89% of patients were diagnosed after 1990 and in total 93% of patients were treated with UDCA. The majority of patients received UDCA therapy in an early stage of the disease and this may be an important contributing factor to the observation that overall mortality in our cohort was similar to the age- and gender-matched background population.

PBC has previously been associated with an increased cancer risk, especially an increased risk of HCC, although evidence has been conflicting. Male gender and unresponsiveness to UDCA therapy seem to be risk factors for HCC in PBC. In the present study, we do not observe an association with any of the reported risk factors including male gender and smoking. While investigating the possible link between PBC and malignancies, several hypotheses arise. The increased HCC risk can be explained by longstanding chronic inflammation resulting in fibrosis and cirrhosis, causing mutations in the cellular machinery. However, for bladder and breast cancer the etiology is less clear. In contrast to HCC, breast and bladder cancer may not be a consequence of PBC, but rather share etiological factors. The striking female predominance in PBC has led to the hypothesis that female sex hormones may trigger disease. Early menarche, late menopause, post menopausal obesity, and hormone replacement therapy (HRT), all leading to increased hormone exposure, are established risk factors for breast cancer. Notably, although risk factors for breast cancer have been studied far more extensively, one large case-control study showed an association between PBC and HRT. Bladder carcinoma occurs in general more often in men than in women, and age
and smoking have been identified as the most important risk factors. Notably, exposure to aromatic amines that can be found in chemical, dye and rubber industries increases the risk of bladder carcinoma.24 In New York, a significant association between a cluster of PBC patients and superfund toxic waste sites contaminated with volatile aromatic hydrocarbons and trichloroethylene was identified.25 Furthermore, frequent nail polish and hair dye use has been linked to PBC, supporting the suggestion that bladder cancer and PBC share etiologic factors.26

There are several limitations to this study including the retrospective design and the large number of missing laboratory tests at time of diagnosis. Although we feel that we have done our utmost to recruit every single patient in a geographically defined area by combining several sources and evaluating medical records on site, there is a chance of missing cases. Serum bilirubin, albumin and AST values at diagnosis were available of 42% of patients only. However, an analysis based on 420 patients is still one of the largest studies described to date and survival analysis showed no significant difference in time until liver transplantation or PBC-related death between patients with and without missing serum values (log-rank p=0.237).

In conclusion, this population-based study showed that PBC is associated with an increased risk of malignancy, especially HCC and bladder cancer. Overall, survival of PBC patients is comparable to the age- and gender-matched general population. Male gender, cigarette smoking, and an elevated bilirubin, decreased albumin, or elevated AST at time of diagnosis, are risk factors for the combined endpoint liver transplantation or PBC-related death.

REFERENCES

Chapter 8


SUMMARY

Primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) are chronic cholestatic liver diseases of unknown etiology. True population-based epidemiological studies are scarce, especially in PSC. There is a need for large population-based studies combining meticulous case-finding and case-ascertainment strategies, which may provide clues as to possible pathogenesis and environmental risk factors for these rare enigmatic diseases. This thesis describes studies on the two largest population-based cohorts of PSC and PBC patients worldwide. The most important findings are depicted below.

Chapter 2 summarises the incidence and prevalence of PSC and PBC between 1972 and 2007. Incidence and prevalence rates of both PSC and PBC vary widely and seem to be increasing. It is unclear whether these are true variations, or whether they reflect methodological differences. Proper epidemiological data may help in identifying etiological factors for these complex diseases. To this end, we aimed to obtain population-based prevalence and incidence figures, in an attempt to gain insight into disease course with regard to survival, liver transplantation, and occurrence of malignancies, as well as risk factors thereof.

Chapter 3 describes a retrospective population-based cohort study. In total, 590 PSC patients were identified in a geographically defined area of the Netherlands comprising almost 8,000,000 inhabitants, showing an incidence of 0.5 and a point prevalence of 6.0 per 100,000. Sixty-eight percent of PSC patients had concomitant inflammatory bowel disease (IBD). The estimated median survival from diagnosis until liver transplantation or PSC-related death in the population-based cohort is 21.2 years, significantly longer than in a liver transplantation PSC cohort. PSC patients have a four-fold increased risk of mortality compared to the general population; however patients with small-duct PSC have a much better survival compared to those with large-duct PSC. In multivariate analysis, age at diagnosis, colorectal carcinoma, and cholangiocarcinoma are risk factors for endpoint PSC-related death. Notably, NSAID use is associated with a decreased risk for the endpoint liver transplantation. PSC patients had a 398-fold increased risk for developing cholangiocarcinoma, and a five-fold increased risk for developing colorectal carcinoma compared to the age- and gender-matched general population. Strikingly, colorectal carcinoma occurred at a much younger age in PSC-IBD patients compared to IBD controls (39 vs. 59 years). Regular colonoscopic surveillance is associated with better outcome.

PSC is strongly associated with IBD. As described in chapter 2, 68% of PSC patients have concomitant IBD in particular ulcerative colitis (UC) or colonic Crohn’s disease (CD). In chapter 4 we aimed to assess the IBD phenotype associated with PSC in a large thoroughly ascertainment and phenotyped PSC cohort, as well as in an age- and gender-matched IBD control group using endoscopic and histopathologic criteria. IBD in PSC patients represents a distinct phenotype in that pancolitis is observed in 94% of PSC-UC and colitis in 96% of PSC-CD patients. Backwash ileitis and rectal sparing were rare findings (<10%) in the cohorts under study.

As described in chapters 2 and 3, IBD is the most important risk factor for developing PSC. PSC and IBD are considered to be complex genetic diseases, meaning that a combination of genes and environmental risk factors cause the disease. In PSC, little is known about these disease-triggering factors. As described in chapter 5 cigarette smoking is associated with a lower risk of developing PSC, independent of the protective effect it has on UC; and previous appendectomy decreased the risk of IBD, but not of PSC. Whether patients were living in cities or in the country, or whether or not they had been infected with reovirus type 3, were not associated with developing PSC.

The recent addition of IgG4-associated cholangitis (IAC) to the spectrum of chronic cholangiopathies has created the clinical need for reliable methods to discriminate between IAC and PSC. Chapter 6 provides an algorithm to distinguish IAC from PSC using serum IgG analyses. Total IgG and IgG subclasses were measured in serum samples of IAC (n=73) and PSC (n=310) patients, as well as in serum samples of disease controls (PBC; n=22). Serum IgG4 levels were elevated above the upper limit of normal (ULN=1.4 g/L) in 15% of PSC patients. The highest specificity and positive predictive value for IAC were reached when applying the 4x ULN (sIgG4 >5.6 g/L) cut-off. However, in patients with a serum IgG4 between 1.4 and 2.8 g/L the PPV of sIgG4 for IAC is only 28%. Using the sIgG4/sIgG1 ratio cut-off 0.24 in the diagnostic algorithm markedly improved PPV and specificity in this subgroup.

By applying a similar case-finding and case-ascertainment strategy as for PSC, we were able to identify all PBC patients in a large geographically defined area of the Netherlands, as depicted in chapter 7. In total, 992 PBC patients were included, resulting in a mean incidence of 1.1 and a point prevalence of 13.2 per 100,000 inhabitants. Incidence and prevalence rates were found to be increasing over time. No geographical differences in disease distribution were observed. No difference was found in smoking behaviour, age at menarche, age at first pregnancy, gravidity, or number of children between cases and controls.

To date, the natural history of PBC had mainly been studied in tertiary referral centres. In order to identify risk factors for development of malignancies and disease progression, we evaluated the disease course of the population-based PBC cohort, as described in chapter 8. Mortality is found to be similar to the age- and gender-matched population. However,
male gender, smoking, elevated bilirubin, decreased albumin, and elevated AST at time of diagnosis, are all associated with an increased risk for the combined endpoint PBC-related death or liver transplantation. PBC patients run a nine-fold increased risk of developing hepatobiliary malignancies, a five-fold increased risk of developing urinary bladder cancer, and a 1.8-fold increased risk of developing breast cancer.
SAMENVATTING voor niet-ingewijden

Primaire scleroserende cholangitis (PSC) en primaire biliaire cirrose (PBC) zijn chronische cholestatische leverziekten van onbekende oorsprong. Cholestase is het onvoldoende afvoeren van gal in de lever en galwegen. Ezelt, onderzoekers hebben geen antwoord gevonden op de vraag hoe deze ziekten zich ontwikkelen. Elke dertig jaar worden nieuwe behandelingen voor deze ziekten gepresenteerd. Echter, populatiegebaseerde studies zijn zeldzaam, vooral naar PSC. Daarom is in 2006 een multicenter studie geïnitieerd in 44 ziekenhuizen in Nederland met als doel om alle PSC- en PBC-patiënten te verzamelen in een geografisch aaneengesloten gebied in Nederland en zo inzicht te krijgen in de epidemiologie en de belangrijkste resultaten en conclusies.

wordt door ontsteking van de gehele dikke darm. Zoals beschreven in hoofdstuk 2 en 3 is IBD de belangrijkste risicofactor voor het ontstaan van PSC. PSC en IBD worden beschouwd als complexe genetische ziekten, wat betekent dat er niet één oorzaak is voor het ontstaan van een ziekte, maar dat een ziekte ontstaat door een combinatie van genen en omgevingsfactoren. Er is weinig bekend over deze ziekte-uitlokkende factoren in PSC.

In hoofdstuk 5 wordt de rol van roken, woonomgeving, eerdere operaties, voorkomen van ziektes in de familie en het doormaken van een infectie uiteengezet. Het is bekend dat roken beschermt tegen het ontwikkelen van colitis ulcerosa. Voor PSC is dit gunstige effect nog sterker, onafhankelijk van het bekende beschermende effect op colitis ulcerosa. Het verwijderen van de blinde darm beschermt tegen het ontwikkelen van IBD, echter niet tegen PSC. Het wonen in een stad of op het platteland is niet van invloed op het ontstaan van PSC.

De recente toevoeging van IgG4-geassocieerde cholangitis (IAC) aan het spectrum van chronische galwegziekten creëerde de klinische behoefte aan betrouwbare methoden om onderscheid te kunnen maken tussen IAC en PSC. In hoofdstuk 6 wordt een algoritme gepresenteerd dat helpt bij de interpretatie van serum IgG4 waarden. Bij 15% van de PSC-patiënten wordt in serum een IgG4-waarde boven de bovengrens van normaal (1,4 g/L) gevonden. De hoogste specificiteit en positief voorspellende waarde voor IAC wordt bereikt als 4x de bovengrens van normaal (IgG4 > 5,6 g/L) gebruikt wordt als afkapwaarde. Echter, in patiënten met een serum IgG4 tussen 1,4 en 2,8 g/L is de positief voorspellende waarde van een verhoogde IgG4-waarde slechts 28%. In deze subgroep is het de moeite waard om IgG1 in serum te bepalen. Zowel de positief voorspellende waarde als de specificiteit verbetert als een IgG4/IgG1 ratio afkapwaarde van 0,24 wordt gebruikt in deze specifieke subgroep.

Met een vergelijkbare zoekstrategie en onderzoeksmethode zoals beschreven voor PSC zijn in hoofdstuk 7 alle PBC-patiënten in een geografisch afgebakend gebied in Nederland in kaart gebracht. In totaal woonden 992 PBC-patiënten in het gebied wat neerkomt op een incidentie van 1,1 en een puntprevalentie van 13,2 per 100.000 inwoners. Opvallend is dat incidentie- en prevalentiecijfers toenemen in de tijd. Wonen in een stedelijke omgeving of in een dunbevolkt gebied heeft geen invloed op het ontstaan van PBC. Roken, leeftijd ten tijde van eerste menstruatie en zwangerschap en het aantal doorgemaakte zwangerschappen zijn niet geassocieerd met het ontstaan van PBC.

Het beloop van PBC was tot nu toe voornamelijk onderzocht in tertiaire verwijscentra. In hoofdstuk 8 is het ziektebeloop van het populatiegebaseerde PBC-cohort bestudeerd. De mortaliteit is vergelijkbaar met de algemene bevolking gemacht voor leeftijd en geslacht. Echter, mannelijk geslacht, roken, een verhoogd bilirubine, verlaagd albumine of een verhoogd ASAT op moment van diagnose zijn geassocieerd met een verhoogde kans om aan PBC te overlijden of een levertransplantaat nodig te hebben. Daarnaast hebben PBC-patiënten een 9x verhoogd risico op het ontwikkelen van leverkanker, een 5x verhoogd risico op blaaskanker en een 1,8x verhoogd risico op borstkanker.

De studies in dit proefschrift hebben geleid tot meer inzicht in het ontstaan en het beloop van PSC en PBC. Echter, veel vragen zijn nog onbeantwoord en blijven dokters en wetenschappers stof tot nadenken geven. De zoektocht naar de oorzaak van PSC en PBC is van groot belang voor de begeleiding en behandeling van duizenden patiënten wereldwijd.
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### PHD PORTFOLIO

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<td>Primary sclerosing cholangitis is associated with pancolitis and not backwash ileitis</td>
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<td>Primary sclerosing cholangitis is associated with pancolitis and not backwash ileitis</td>
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<td>PSC-IBD: a distinct clinical phenotype</td>
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<td>Superior transplant-free survival in a large population-based cohort of patients with primary sclerosing cholangitis</td>
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<td>B.D. van Rhijn, Epidemiology, disease spectrum and burden of inflammatory bowel disease in the Netherlands</td>
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<td>E. Karregat, Epidemiology, disease spectrum and burden of Inflammatory bowel disease in the Netherlands</td>
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<td>R. Bokelaar, Primary biliary cirrhosis and environmental involvement: a pilot study in the Amersfoort region</td>
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DANKWOORD

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Ulrich - secur en genuanceerd
Anje - geduldig en creatief

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Esther, Caroline en Sander - gidsen in een wondere wereld

Simone en Charlotte - sterke vrouwen en onnavolgbare multitaskers
Liesbeth - enthousiast en energiek

Noortje - zon op links
Maaike - geboren paranimf

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