

UNIVERSITA' DEGLI STUDI DI MILANO
FACULTY OF MEDICINE AND SURGERY
RESEARCH DOCTORATE IN GASTROENTEROLOGY
CYCLE XXVII

**PAEDIATRIC ONSET PRIMARY SCLEROSING
CHOLANGITIS: CLINICAL COURSE AND OUTCOME**

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Academic Year 2014

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ACKNOWLEDGEMENTS

Alla mia famiglia, agli amici e ai colleghi di Milano e di Helsinki

To my family, to my friends, to my colleagues from Milan and Helsinki

Sukulleni, ystäväilleni, kollegilleni Milanosta ja Helsingista

ABBREVIATIONS

AIH: autoimmune hepatitis

ALK: alkaline phosphatase

ALT: alanine aminotransferase

AMA: anti-mitochondrial antibodies

ANCA: anti-neutrophil cytoplasmic antibodies

ANNA: anti-neutrophil nucleus antibodies

AST: aspartate aminotransferase

CA 19-9: carbohydrate antigen 19-9

CT: computed tomography

DIA: digital imaging analysis

ERC: endoscopic retrograde cholangiography

FISH: fluorescence in situ hybridisation

GGT: gamma glutamyltranspeptidase

IBD: inflammatory bowel disease

IgG: immunoglobulin G

IgM: immunoglobulin M

INR: international normalized ratio

LKM1: Liver-Kidney Microsomal antibodies type 1

MRCP: magnetic resonance cholangio-pancreatography

MRI: magnetic resonance imaging

PSC: primary sclerosing cholangitis

SMA: anti-smooth muscle antibodies

LT: liver transplantation

UDCA: ursodeoxycholic acid

US: ultrasound

ABSTRACT

BACKGROUND AND AIMS

The natural history of paediatric onset PSC and PSC/AIH overlap syndrome is poorly known. Thus, this study was aimed at evaluating the clinical course and outcome of patients with a paediatric onset of disease.

MATERIALS AND METHODS

Between December 1993 and December 2011, thirty-five patients (median age at diagnosis 15 years), with confirmed PSC by cholangiography (ERC) and PSC/AIH by cholangiography and the modified score for AIH, were traced at Helsinki University Central Hospital. Clinical characteristics (symptoms and signs, associated diseases), diagnostic procedures (lab tests, liver biopsy, ERC) and long-term follow-up (mortality, liver transplantation, recurrence of the disease in the graft, malignancy) were reassessed until December 2013.

RESULTS

The original diagnosis was PSC in 22 children (63%) and PSC/AIH overlap syndrome in 13 (37%). At diagnosis most of the children had an insidious onset and most an associated IBD, being UC the most common form. Still, 4/35 (11%) had cirrhosis. Endoscopic retrograde cholangiography during follow-up was available for all of the patients and images showed a progression of intra-hepatic disease in 13/28 (46.4%) ($p=0.0102$). In the last follow-up (median 8 years), all patients were alive. Twenty-eight out of 35 patients (80%) and 13/35 patients (40%) were taken UDCA and immunosuppressive therapy, respectively; 3 patients were without treatment. Transaminases and GGT improved significantly. Four patients (11%) had undergone liver transplantation (after a median 7.5 years) and one was listed; and additional patient was transplanted because of Budd-Chiari syndrome. No difference in graft free survival was seen between patients with PSC and PSC/AIH. Three patients (two with

PSC and one with PSC/AIH overlap syndrome) presented with cirrhosis. No malignancy occurred.

CONCLUSION

The clinical outcome of primary sclerosing cholangitis and overlap syndrome seems comparable including their progression to cirrhosis and requirement for liver transplantation.

INTRODUCTION

PRIMARY SCLEROSING CHOLANGITIS

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by inflammation and progressive fibrosis of intra- and extra-hepatic bile ducts (1). In children PSC is often associated with features of autoimmune hepatitis (AIH); this peculiar form of PSC is referred as **PSC/AIH overlap syndrome** (2). Paediatric onset PSC is a rare condition, while the clinical course and prognosis of the disease is still unclear. To our knowledge, so far only four papers with a long follow-up (one from Europe and three from U.S.A.) have been reported the clinical course and prognosis of paediatric onset PSC (3-6) and new studies on this topic are warranted (7).

This thesis was conducted thanks to the collaboration between “Meilahti Hospital - Helsinki University” (Helsinki, Finland) and “Fondazione IRCCS “Ca’ Granda” Ospedale Maggiore Policlinico – University of Milan” (Milan, Italy). Firstly, the introduction reports an overview of PSC, regarding particularly epidemiology, aetio-pathogenesis, diagnosis and therapy of the disease and highlighting the main differences between children and adults. Secondly, the study presents the long-term clinical course and prognosis of a paediatric onset PSC and PSC/AIH overlap syndrome in a cohort of patients in Finland.

DEFINITIONS

PSC is a chronic cholestatic liver disease characterized by inflammation and fibrosis of intra- and extra-hepatic biliary ducts, leading to focal or multiple bile ducts strictures (8, 9). The disease was first described by Hofmman in 1867. No medical treatment is currently available and the disease usually progresses to cirrhosis, liver failure and need of liver transplantation

(LT). In children, adolescent and young adults, PSC is often associated with clinical, biochemical and histologic features of AIH and this clinical variant is referred as **autoimmune sclerosing cholangitis** or **PSC/AIH overlap syndrome** (2). **Small duct PSC** refers to a clinical variant of PSC characterized by similar cholestatic and histologic features of PSC but with a normal cholangiographic picture because the disease involves the smallest bile ducts (10).

The term **secondary sclerosing cholangitis** refers to a group of diseases causing patchy inflammation, fibrosis and strictures of the intra- and/or extra-hepatic biliary system and leading cholestasis (11). **Immunoglobulin G4 (IgG4) associated cholangitis** may represent a distinct entity characterized by the same cholangiographic features of PSC but with important differences such as high levels of IgG4 in serum, association with other fibrotic diseases (e.g., autoimmune pancreatitis) and good response to steroid therapy (12).

EPIDEMIOLOGY

Incidence and prevalence. The epidemiology of PSC is not well-characterized as few population-based studies have been published on this topic and only one focused on paediatric population (5). This study was conducted in the State of Utah (U.S.A.) in 29 children with PSC and 12 children with PSC/AIH, reporting an incidence and prevalence of PSC as 0.2/100.000 inhabitants/year and 1.5/100.000 inhabitants, respectively and of PSC/AIH as 0.1/100.000 inhabitants/year and 0.6 /100.000 inhabitants, respectively (5). Another population-based study (13) was conducted in the State of Alberta (Canada) including 46 adults and only three children. The reported incidence rate for PSC in children was 0.23/100.000 inhabitants per year; no data regarding prevalence has been shown. No other studies have investigated the prevalence of PSC and the incidence and prevalence of PSC/AIH overlap syndrome in paediatric population.

Recently, one systematic review and meta-analysis conducted in Canada (14) and one systematic review conducted in Netherland (15) presented a comprehensive overview of the epidemiology of PSC. These systematic reviews have been included studies conducted mostly in adults. The first systematic review, including six population-based studies (two from North America and four from Europe) (13, 16-19) reported an incidence rate of 1.0/100.000 inhabitants per year, a risk two-fold greater in males and a median age at the diagnosis of 41 years; PSC incidence rate seemed to increase over time. The second systematic review included eleven studies of which only four (13, 16, 19, 20) were considered of good quality according to the definition of study population, case-finding method and case-ascertainment criteria. The incidence and prevalence rate for PSC ranged from 0 to 1.3/100.000 inhabitants per year and 0 - 16.2/100.000 inhabitants, respectively. PSC incidence showed an increasing in temporal trend (15).

Inflammatory bowel disease (IBD) - PSC. PSC is strongly associated with IBD in children as well as in adults. In children, IBD is reported in about 80% of cases (mostly ulcerative colitis) (6). IBD seems to be less common in children affected by PSC/AIH (2). Conversely, in a population based-study PSC occurred in 9.9% and 0.6% of children with ulcerative colitis and Crohn's disease, respectively and PSC/AIH in 2.3% and 0.4% of children with ulcerative colitis and Crohn's disease, respectively (5).

In adults, IBD is reported up to 80% of PSC patients in northern countries (i.e., North America and Scandinavia) compared to 20-54% of those living in South Europe and Asia (21-24); ulcerative colitis accounts for the majority (80%) of cases. The prevalence of PSC in IBD adult patients is 2-7.5% in ulcerative colitis and 1.4-3.4% in patients with Crohn's disease (25, 26).

IBD-PSC shows a unique phenotype (27). The inflammation is usually mild (28) and the distribution is typically pancolonic, with rectal sparing, with frequent backwash ileitis (27).

PSC-IBD patients less likely undergo colectomy but they have more frequently pouchitis after surgery compared to patients with only IBD (27, 29).

AETIOPATHOGENESIS

The aetiopathogenesis of PSC and PSC/AIH is unknown. Whether or not PSC/AIH is a distinct disease entity or a result of progression from PSC to AIH or *vice versa* is still debated (30, 31). The higher incidence of these diseases in northern countries (i.e., North America and North Europe) as well as in patients affected by IBD suggests an interaction between unidentified environmental risk factors and a complex genetic background.

Environmental risk factors. Studies on environmental risk factors in paediatric onset PSC and PSC/AIH overlap syndrome are still lacking in the literature. So far, five studies on environmental risk factors in adults have been published. A suggested protective role of smoking (32-34) and of tonsillectomy (35) is reported. Appendectomy is not a protective factor for PSC (33, 35) as it is for ulcerative colitis. Finally, coffee consumption and females hormones might have a protective role against PSC (34).

Genetic factors. First-degree relatives of patients with PSC have a 100-fold increased risk of developing disease, suggesting a strong genetic component (36) that seems to include both non-HLA (37, 38) and HLA genes on chromosome 6 (39). Genes may be involved in cholangiocyte biology, inflammation pathway, fibrosis and carcinogenesis. The largest genetic study conducted recently on PSC patients showed that half of the PSC genes are not associated with IBD but they overlap with other autoimmune diseases such as psoriasis, rheumatoid arthritis, coeliac disease and diabetes I (40).

Others factors. Another hypothesis involves **immunologic mechanisms**. Inflamed gut (IBD) may release TNF α that in turn increases methylamine in portal blood that via VAP1 activity might induce MADCAM1, an endothelial adhesion molecule usually absent from liver

vascular bed. In turn, this aberrant MADCAM1 may recruit lymphocytes in the liver (hepatic homing) (41, 42). Another hypothesis involves the loss of hydrogencarbonate layer of normal biliary epithelium, which usually protects against the biliary acid (**bile duct toxicity**) (43). A change in gut microbiota may promote penetration of microbes or toxins from colon to the liver via-portal flow. A specific genetic background (i.e., FUT2) may be associated with **bacterial or viral infection** (44).

DIAGNOSIS

Symptoms and signs. Most of the children with PSC and PSC/AIH are referred for nonspecific complaints such as fatigue, abdominal pain, isolated splenomegaly/hepatomegaly and jaundice (45). Itching is rare (less than 20% of cases) (4, 6). At the disease onset about 19-29% of children is asymptomatic (4, 6), presenting severe fibrosis or cirrhosis on liver histology (4). By contrast, in adults asymptomatic patients are over 40% (46). Itching is more common (30%) and other symptoms are fatigue, abdominal pain, hepatomegaly/splenomegaly (46, 47). Cirrhosis and its complications at PSC onset are more rare in adults (46, 47).

Laboratory tests. Children with PSC or PSC/AIH usually present elevation of transaminase (alanine aminotransferase - ALT and aspartate aminotransferase - AST) and gamma glutamyltranspeptidase (GGT) (4, 6). Transaminases might be higher in children affected by PSC/AIH (5, 48). Total bilirubin and alkaline phosphatase (ALP) might be within normal range in the majority of the patients (4-6); hepatic iso-enzyme of ALP would be a more accurate marker, as the normal range of ALP is large due to the effect of bone maturation in children. By contrast, elevation of ALP is the hallmark of PSC in adults; transaminases are usually 2-3 times upper normal limit (46, 47). Also in adult total bilirubin level is within normal range in most of the patients (46, 47).

Serology. A large number of autoantibodies have been associated with PSC without any difference between paediatric and adult patients. Anti-Neutrophil Cytoplasmic Antibodies (ANCA) were detected in PSC patients by Snook et al in 1989 (49). The peri-nuclear pattern (pANCA) is the most common being reported in 26-94% of the patients (50, 51). However, pANCA present an atypical pattern in PSC as the main antigen is located in the nucleus rather than in cytoplasm and thus they are referred as Anti-Neutrophil Nucleus Antibodies (ANNA) (51). pANCA have a low specificity for PSC being present also in AIH, IBD and other autoimmune diseases (51) and they are not accurate in differentiating between PSC and PSC/AIH in children (4-6). Currently, pANCA should not be used in the diagnosis and follow-up of patients affected by PSC (9). Anti-Nuclear Antibodies (ANA) and Anti-Smooth Muscle Antibodies (SMA) are the hallmarks of AIH type I both in children and in adults (3, 52). However, ANA and SMA are also detected in 8-77% and 0-83% of the PSC patients, respectively (53) and in more than 90% of children with PSC/AIH often in association with an increase level of Immunoglobulin G (IgG)/gamma-globulins (2, 4-6). Therefore, ANA and SMA should not be checked routinely in patients with PSC but only in those with a suspicious of overlap with AIH (9). Anti-Mitochondrial Antibodies (AMA) used in the diagnosis of primary biliary cirrhosis and anti-Liver-Kidney Microsomal antibodies type 1 (anti-LKM1) used in the diagnosis of AIH type II have never been detected in patients with PSC or PSC/AIH (53).

Liver Biopsy. Liver biopsy should not be performed routinely in the presence of an abnormal cholangiogram because it doesn't add useful diagnostic information and the rate of false negatives is high (54). However, in some Centres liver biopsy is performed routinely in children to exclude PSC/AIH overlap syndrome. Peri-ductal concentric fibrosis ("onion-skin"), the classic histopathologic finding of PSC, is uncommon (about 13% of the patients) (54). In two studies on paediatric onset PSC liver biopsy was performed in almost all the

patients at the time of diagnosis, showing features highly suggestive of PSC in the majority of them (4, 6). However, liver biopsy is recommended only in suspected small duct PSC or overlap syndrome (8, 9).

Imaging. Traditionally, endoscopic retrograde cholangiography (ERC) has been considered the gold standard for the diagnosis of PSC but its role is now debated (8, 9). The cholangiographic pattern of PSC includes short and annular strictures, alternating with normal or slightly dilated segments (“beaded appearance”) diffusely involving both the intra- and extra-hepatic bile ducts; about 25% of patients may have only intra-hepatic disease whereas isolated extra-hepatic disease is extremely rare (< 5%) (55). The same findings are reported in children (4, 6, 45). However, ERC needs hospitalization and the technique is associated with complications also in PSC patients with an overall rate of 10% (56, 57). The two main ERC-related complications reported in PSC patients are acute pancreatitis (57) and acute cholangitis (56). Magnetic resonance cholangio-pancreatography (MRCP) has been suggested as an alternative option to ERC because of cost-effectiveness (58), absence of ionising radiations and complications. Still, a recent meta-analysis on diagnostic performance of MRCP for diagnosis of PSC, including 6 studies (59-63), reported a sensitivity of 0.86 and a specificity of 0.94 with a positive likelihood ratio of 15.3 and a negative likelihood ratio of 0.15 (64). For the considerations mentioned-above, MRCP should be used in the diagnostic workup of autoimmune liver diseases in children (7, 61, 65). However, the studies on MRCP performance in diagnosing PSC have many limitations: the study designs, the absence of clear gold standard, the low number of patients included and the lack of stratification according to disease severity. Moreover, MRCP was not so accurate as ERC in detection of early changes of PSC in some studies (59, 66). Therefore, ERC should have still a pivotal role in PSC management both in diagnosis (i.e., monitoring of biliary dysplasia with brush cytology and DNA analysis, see below) and in therapeutic approach (i.e., biliary dilatation and stenting).

DIFFERENTIAL DIAGNOSIS

PSC should be differentiated from:

Small duct PSC. It is a clinical variant of PSC, diagnosed in a patient with normal cholangiography but with similar cholestatic and histological features of PSC (67, 68). In one paediatric series small duct PSC was reported in about 35% of the patients (4). Recently, one study (10) investigated the natural history and long-term prognosis of a cohort of small duct PSC patients (n=83) compared with classic PSC; this cohort includes three different series of small duct PSC patients published in 2002 (69-71). This variant is more prevalent in male (male:female=2:1) and the median age at diagnosis is 38 years (IQR: 28-50) like in classic PSC. About 80% of patients have IBD (mostly UC) like in classic PSC. Interestingly, about 30% of the patients' progresses to large duct PSC in a medium time of 7.4 years (5.1-14); nevertheless patients may progress to end-stage liver disease and need of LT without developing necessarily large duct PSC. LT free survival is longer in small duct PSC patients (13 years) than in classic PSC (10 years) and the disease might recur in the allograft. Finally, patients affected by small duct PSC seem to have a better long-term prognosis (mortality was 13% compared to 30% in large duct PSC patients); neither cholangio-carcinoma nor hepatocellular-carcinoma is reported. Ursodeoxycholic acid (UDCA) does not seem to be an effective treatment.

Immunoglobulin G4 associated cholangitis. It is a steroid responsive inflammatory biliary disease, frequently involving extra-hepatic biliary tree and often associated with other fibrotic disorders (e.g., autoimmune pancreatitis) (12, 72). It is characterized by elevation of IgG4 in serum and by infiltration of IgG4 positive plasma cells in the organs involved (12). Very limited data are available on the disease but it seems that it does not occur in children (4, 6).

Secondary sclerosing cholangitis. Before a diagnosis of PSC is given, secondary causes of sclerosing cholangitis should be properly excluded. The main causes are: biliary stones, Caroli's disease, biliary surgery, cholangiocarcinoma, chronic biliary infections, drugs, and ischemia. The diagnostic criteria are described in a recent review and are not reported in this dissertation (11).

MALIGNANCY

The increased risk for gastrointestinal malignancy in patients with PSC is well established. In a Swedish cohort of 604 PSC patients the standard incidence ratio for all gastrointestinal cancers combined was 28.6 (95%CI: 22.4-36.1); still the standard incidence ratio was 160.6 (95%CI: 120.3-210.1) for hepatobiliary cancer (i.e., cholangiocarcinoma, hepatocellular carcinoma and gallbladder carcinoma), 10.3 (95%CI: 5.3-18.1) for colorectal cancer and 14.3 (95%CI: 4.7-33.4) for pancreatic cancer (73).

Cholangiocarcinoma. Cholangiocarcinoma did not occur in two series of PSC children after a median/mean follow-up of 6.9/6.5 years (4, 6). In a population-based study in children, cholangiocarcinoma occurred in 2/29 PSC patients (6.9%) and both of them died (5). In population-based studies in adults the cumulative incidence of cholangiocarcinoma has been reported between 7-14% (46, 47, 73-76). Up to 50% of cholangiocarcinoma are diagnosed within the first year of diagnosis of PSC (73, 74) and thereafter the yearly incidence is 0.5-1.5% (73, 75, 76). The diagnosis of cholangiocarcinoma in PSC patients is based on tumour markers in serum, imaging modalities, biliary brush cytology and analysis of DNA but in 37% of patients it is diagnosed during intended liver transplantation or autopsy (74). In the Mayo Clinic study carbohydrate antigen 19-9 (CA19-9) had an optimal cut-off of 20 U/mL (normal value < 40 U/mL), which yielded a positive predictive value of 23% and a negative predictive value of 96% for the diagnosis of cholangiocarcinoma (76). There is no study

showing a role of CA19-9 as screening test in asymptomatic PSC patients, although a screening strategy combining serum CA19-9 and imaging study has been suggested (8, 76). In the Mayo Clinic study US, CT and MRI had a poor positive predictive value (48%, 38% and 40%, respectively) and a high negative predictive value (95%, 95%, 91%, respectively) for cholangiocarcinoma; the positive predictive value improved when overall finding was considered (i.e., well-defined mass lesion) (76). Brush cytology has a sensitivity of only 8% but an excellent specificity (80-90%) for detection of low- and high-grade dysplasia (76); however this method needs to be validated in the future and ERCP with brush cytology should not be considered as surveillance for cholangiocarcinoma (8, 9). Finally, in the Mayo Clinic study digital imaging analysis (DIA) and fluorescence in situ hybridisation (FISH) had an high negative predictive value (87% and 95%, respectively) and a low positive predictive value (50% and 52%, respectively) for chromosomal abnormalities (and these techniques may increase the diagnostic performance of brush cytology (76).

Colorectal carcinoma. Colorectal carcinoma did not occur in three series of PSC children after a long follow-up (2, 4, 6). However, PSC has been reported as a risk factor for IBD-related colorectal cancer (77). A meta-analysis, including eleven studies in adults, concluded that the risk of dysplasia/colorectal cancer in PSC-UC patients is increased then UC patients alone with an OR of 4.7 (95%CI: 3.6-6.4) (78). A high risk was reported also for CD (79). The tumour seems to develop more often on the right side of the colon although it can also be located on the left side (80-82). The mechanism by which PSC could be associated with an increased risk of colorectal cancer in IBD is still unclear. PSC-IBD patients usually have pancolitis with a mild disease activity, which could be involved in cancer development (27). Colorectal cancer can also occur after LT (83-85). Finally, a chemoprevention effect of UDCA has been suggested but the evidence of its efficacy is still limited. Surveillance

colonoscopy should be performed at one-year to 2-year from the time of diagnosis of PSC; this interval could be longer in children (8, 9).

Others tumours: PSC patients have a high risk of gallbladder cancer (86); this risk seems to be absent in children (4-6). However, yearly US of gallbladder is recommended and cholecystectomy should be performed in presence of polyps (8, 9).

PSC patients with cirrhosis may develop hepatocellular carcinoma and they should be managed according to general guidelines (8, 9).

TREATMENT

UDCA. In adults the European Association for the Study of the Liver reports “The limited data base does not yet allow a specific recommendation for the general use of UDCA in PSC”, whereas the American Association for the Study of Liver Diseases recommends “against the use of UDCA as medical therapy” (8, 9). Initial studies demonstrated that UDCA improves only biochemical parameters and histologic features (87-89); a double blind randomised placebo-controlled study conducted in 105 patients for a median follow-up of 2.2 years showed that 13-15 mg/kg of UDCA per day improves biochemical parameters but no survival (90). Subsequently, studies in adults showed that increased doses of UDCA (17-23 mg/kg per day) improve biochemical parameters but not significant end-points like cirrhosis, need of liver transplantation and death (91, 92). Recently, a long-term double blind randomised placebo-controlled study conducted in 150 adult patients with PSC was terminated after 6 years because of high rate of adverse events (i.e., cirrhosis, cholangiocarcinoma, liver transplantation and death) in UDCA group (93); interestingly adverse events are higher in patients with early stage disease and normal biochemical parameters (94). Finally, UDCA does not seem to prevent cholangiocarcinoma and colorectal cancer as previously reported (95-97). Only few retrospective series conducted in paediatric

patients reported that also in children UDCA seems to improve clinical and biochemical features of the patients without having an impact on survival (4, 6, 48).

Immunosuppression. Prednisolone, budesonide, azathioprine, cyclosporine, methotrexate, mycophenolate and tacrolimus have not effect on PSC (8, 9). However, corticosteroids and azathioprine may have a role in children with PSC/AIH overlap syndrome (2), although the real impact on survival is still lacking (4, 6) as immunosuppressive therapy reduces the inflammation component (i.e., interface hepatitis) without any improvement on biliary changes.

Endoscopic treatment. Endoscopic treatment is needed in patients with a symptomatic dominant stricture (i.e., in presence of jaundice, itching, right upper quadrant abdominal pain, cholangitis, worsening of biochemical indices) (8, 9). A dominant stricture is defined as a stenosis with a diameter of ≤ 1.5 mm in the common bile duct or of ≤ 1 mm in the hepatic duct (98). A dominant stricture occurs in about 50% of PSC patients during the follow-up (47, 98); the exact prevalence in children with PSC is unknown but probably is low. The appearance of a dominant stricture always raises the suspicion of cholangiocarcinoma and brush cytology and/or biopsy should always be obtained.

So far, no randomized controlled trial on the efficacy and optimal method of endoscopic therapy in the treatment of dominant stricture in PSC has been published. However, two retrospective studies suggested that endoscopic therapy improves survival in symptomatic PSC patients and it is associated with a low complication rate (99, 100). Sphincterotomy is used to facilitate balloon dilatation and/or stenting and it is rarely used alone (100, 101). Balloon dilatation has been shown to be effective both with and without stenting (102). However, biliary stenting seems to be associated with more complications and a longest hospitalization than balloon dilatation only (101-104). A multicentre, prospective, randomized interventional trial aim at comparing the efficacy of a single session balloon dilatation and a

short-term stenting is now ongoing in the International PSC Study Group (<http://www.ipscsg.org/>). Percutaneous approach is effective as endoscopic ones but it is associated with increased morbidity (102, 103). Antibiotic prophylaxis before endoscopic and/or percutaneous approach (e.g., ciprofloxacin) is mandatory. Surgery (i.e., cholangioenterostomy or resection of the extrahepatic biliary stricture and Roux Y hepaticojejunostomy) is rarely used and it should be proposed to patients without cirrhosis when endoscopic and percutaneous approach is failed.

Liver Transplantation. LT is the only curative therapy for PSC. Indications for LT did not differ from those of other forms of chronic liver diseases. Model for End-stage Liver Disease score is used for organ allocation in United States and in other countries. Additional indications for LT are intractable pruritus, recurrent bacterial cholangitis and repeatedly confirmed high-grade biliary dysplasia (105). Cholangiocarcinoma is usually a contraindication for LT. Five-year survival after LT is approximately 85% in adults (106, 107) and it could be even higher (90-100%) in children (2, 4, 6). Disease recurrence may occur both in children (2-54%) (2, 4, 6, 108) and in adults (in about 20-25% of cases) (106, 109, 110). Risk factors for recurrence are male sex, presence of an intact colon, active IBD and history of acute cellular rejection (111, 112); younger age (< 30 years), cytomegalovirus infection within three months after LT and graft from related donors are also reported in children (108). The impact of PSC recurrence on survival remains still controversial (109, 112, 113).

AIM

The aim of the present study was to report a long-term clinical course and outcome of a cohort of patients with a paediatric onset of PSC and PSC/AIH in order to contribute to a better definition of the natural history and management of these conditions.

MATERIAL AND METHODS

Study design

Retrospective observational cohort study.

Study area, period and population

Between December 1993 and December 2011, 41 patients with a suspected paediatric onset PSC and PSC/AIH were consecutively referred to the HUCH, a tertiary referral centre in Finland (5.4 million inhabitants), serving a defined population of about 1 500 000 inhabitants and covering approximately 30% of the total child population in the country (www.stat.fi). Most of the endoscopic retrograde cholangiography investigations in paediatric and adult PSC patients are performed at HUCH.

Case-ascertainment

One gastroenterologist and one paediatrician reviewed all the patients' medical records to ensure the correct diagnosis. Diagnosis of PSC was based on: (i) typical imaging features of PSC in ERC, and eventually (ii) presence of cholestasis (i.e., elevated gamma GGT and/or ALP), and (iii) liver histology suggestive or typical for PSC. Patients affected by secondary sclerosing cholangitis, as well as by other liver diseases (i.e., viral and/or metabolic), were excluded. Patients fulfilling the diagnostic criteria for both PSC and AIH were considered as having PSC/AIH. To this respect, the simplified diagnostic criteria for AIH, recently validated in children (114), were retrospectively applied to the patients to ensure the diagnosis of PSC/AIH.

Data regarding clinical history (i.e., symptoms and signs at the time of diagnosis and in the last follow-up, associated IBD, associated autoimmune diseases) were collected. Symptoms and signs of the disease onset were grouped with the following criteria: asymptomatic (e.g. elevation of liver enzymes discovered by chance), acute hepatitis (e.g., malaise, fever, nausea and vomiting, diarrhoea, abdominal pain followed by jaundice and cholestasis), complications

of portal hypertension (e.g., encephalopathy, ascites, oesophageal varices, splenomegaly) and acute liver failure. Laboratory tests at the time of diagnosis and during the last follow-up were collected. Biochemical parameters included total bilirubin, ALT, AST, GGT, ALP, albumin, and international normalized ratio (INR). Serologic parameters were also collected, including immunoglobulin M (IgM), IgG, ANA, SMA, AMA, anti-LKM1 and pANCA. Medical treatment during the follow-up time (i.e., UDCA and immunosuppressive) was detected. Finally, need and timing of LT, as well as occurrence of complications associated with PSC (i.e., cholangiocarcinoma, colorectal carcinoma) were registered.

Liver biopsies were re-reviewed by an experienced pathologist blinded to the patients' clinical history. The pathologist looked for the following features: number of portal spaces included in the samples, peri-cholangiolar fibrosis, fibrosis (staging), inflammation (grading), portal inflammation, interface inflammation, lobular inflammation, rosette formation, plasma cells, eosinophil, granuloma, cholangitis, peri-cholangitis, necrosis, macro- and micro-steatosis, copper (when available), diastase PAS positive globules (when available), hepatocellular cytokeratin 7 positivity (when available), loss of bile ducts, acute cholestasis, and PSC alteration (0-3; 0=no features of PSC, 1=unspecific features of PSC, 2=features suggestive of PSC and 3=diagnostic features of PSC). Some of these features were detected in order to exclude other liver diseases.

In our hospital, all the patients with a suspicion of PSC undergo ERC. PSC changes detected by cholangiography are scored with Amsterdam PSC score (115), modified regarding intrahepatic scoring at our Clinic (Table 1). During ERC, brush cytology is performed from both intra- and extra-hepatic bile ducts and an additional sample for DNA flow-cytometry is collected only when the biliary changes are moderate or severe according to the modified Amsterdam Score (score > 2). Depending on the modified Amsterdam PSC score and presence of dysplasia and/or aneuploidy, the patient is followed with ERC and abdominal CT

or MRI. In case of severe dysplasia repeatedly confirmed on brush cytology, the patient is considered as a candidate for LT. All ERC images were re-reviewed independently by two experienced gastroenterologists. The cholangiographic findings including focal strictures, focal dilatations, and any alterations of the bile ducts wall involving both intra- and/or extra-hepatic bile ducts were re-scored by using the modified Amsterdam PSC score. All the therapeutic procedures performed during ERC (i.e., sphincterotomy, biliary dilatation, and/or stenting) were also recorded.

For the study purpose, follow-up was considered to start when the patient was referred to HUCH for the suspicion of PSC (liver biopsy and/or ERC) and ended at the time of the last consultation at HUCH (outpatient clinic and/or liver biopsy and/or ERC by the end of December 2013).

Statistical analysis

Data are presented as median and range when continuous. Since the normal range of ALP varies according to the age of the paediatric patient, ALP was considered a categorical variable (i.e. normal/elevated).

PSC and PSC/AIH groups were compared with the patients assigned to their original diagnosis and with the patients assigned to their reviewed diagnosis. Categorical variables were compared between and within groups by using Fisher's Exact Test (small number of subjects). Continuous variables were compared between and within groups by using Mann-Whitney Test and Wilcoxon Test, respectively. Kaplan-Meier method was performed for survival analysis; the end-points were LT and/or listing for LT for PSC and/or PSC/AIH. A p value < 0.05 was considered statistically significant.

Ethical consideration

The study protocol has been approved by the Ethics Committees of HUCH (Diary number: 64/13/03/03/12).

RESULTS

Patient population

In the final analysis 35 patients (median age at diagnosis 15 years) were included (Table 2). Five patients (median age 14 years: range 7-17; male: 1) were excluded because the diagnosis has been changed during the follow-up: choledochal cyst in two, hepatitis related to azathioprine in one, acute hepatitis of unknown origin in one and autoimmune hepatitis in one. One patient (10-year-old boy) with a diagnosis of PSC was excluded because he was lost to follow-up.

The original diagnosis was PSC in 22 patients (62.8%) and PSC/AIH in 13 (37.1%) patients. All the cholangiography images at the time of diagnosis were available and review confirmed the diagnosis of PSC in all 35 patients. The simplified score for AIH could retrospectively be calculated in 25 patients (71.4%): 18 patients had their diagnosis confirmed (PSC n=11 and PSC/AIH n=7), whereas in 6 patients the diagnosis was changed on review from PSC into PSC/AIH (probable n= 1 and definite n=5), and in one from PSC/AIH into PSC. In the remaining 10 patients, the simplified score for AIH reassessment could not be calculated because of missing laboratory tests and/or liver biopsy at the time of diagnosis.

Clinical presentation at the time of diagnosis

Background characteristics and clinical presentation of children at PSC and PSC/AIH onset are shown in Table 2. PSC and PSC/AIH patients did not differ regarding sex or age. Asymptomatic elevation of liver enzymes was the most common presentation in both diagnostic groups. None of the patients presented with acute liver failure. At the time of diagnosis, 4/35 patients (11.4%) – 2/22 with PSC (9.1%) and 2/13 with PSC/AIH (15.8%) ($p=0.62$) - were cirrhotic; in one patient the diagnosis was based on clinical and radiologic findings whereas in three on histologic picture. Three of cirrhotic patients had signs of portal hypertension (hepato-splenomegaly). According to the reviewed diagnosis 1/12 patient with

PSC (8.3%) and 3/13 with PSC/AIH (23.1%) were cirrhotic ($p=0.59$). Patients with PSC most likely had an associated IBD ($p=0.006$) and UC was the most prevalent form without being significant ($p=0.06$) (Table 2); the difference was not evident in patients with reassessed diagnosis ($p=0.32$ and $p=0.07$, respectively). Other autoimmune diseases were associated in 3 patients with PSC (diabetes type I, nephritis and leuko-trombocytopenia, respectively) and in 3 patients with PSC/AIH (systemic lupus erythematosus, diabetes type I and rheumatoid arthritis, respectively) ($p=0.65$) and no significant group difference was seen upon reassessment ($p=1.0$).

ERC findings at the time of diagnosis and in the last follow-up

At the time of diagnosis, ten patients (28.6%) had intra- and extra-hepatic disease, 25 patients (71.4%) had only intra-hepatic disease; isolated extra-hepatic disease was not present in any patients. Short papillotomy was performed in 15 (42%) procedures. Dilatation of bile ducts was performed in 9 patients (26%): 3 in patients with intra-hepatic and 6 in patients with extra-hepatic disease. Biliary stenting was not performed on any of the patients.

During follow-up, a repeated ERC was available in 28/35 patients (80%). The median number of ERC per patient performed during the follow-up period was 3 (range 1-8). The modified Amsterdam score showed a progression of intra-hepatic disease in 13/28 patients (46.4%; $p=0.0102$) and of extra-hepatic disease in 3/28 patients (10.7%; $p=0.8516$).

Liver histology at the time of diagnosis

Liver biopsy was performed at the time of diagnosis in 33/35 patients (94.3%). For reviewing, 25/33 (75.7%) samples were available. Of these, the diagnostic liver biopsy could not be re-evaluated in one PSC patient because of the absence of portal spaces in the sample, nevertheless another biopsy performed during the follow-up was reviewed and it showed PSC changes without characteristics of AIH (e.g., interface hepatitis). Histologic features on liver biopsy of children at PSC or PSC/AIH onset after the re-assessment of the diagnosis are

shown in Table 3. Interestingly, 17/35 (48%) of the patients had no features or unspecific features of PSC. Still, 7/35 (20%) had severe fibrosis or cirrhosis at the disease onset.

Laboratory tests at the time of diagnosis and in the last follow-up

The laboratory tests of children at PSC and PSC/AIH onset are shown in Table 4. ALT and/or AST were elevated in 31/34 (91.2%) and in 27/34 (79.4%) of the tested patients, and levels were similar between the groups. Bilirubin was normal in 25/34 (73.5%) and the level was higher in the PSC/AIH group ($p=0.0430$), although the median was within the reference range. GGT was elevated in 30/34 (88.2%) and no difference between groups was found. ALP was normal in 12/34 (35.3%) of the patients. Albumin was lower in the PSC/AIH group ($p=0.0110$), whereas INR was within the normal range for all patients. Regarding serological parameters, IgG was elevated in 24/33 (72.7%) and the level was higher in the PSC/AIH group ($p=0.0352$); by contrast IgM level was within normal range in all of the tested patients. ANA and/or SMA titre was positive more frequently in the PSC/AIH group ($p=0.0248$), whereas the rate of pANCA positive patients did not differ between groups. Finally, AMA and anti-LKM1 were negative in all tested patients (n=34 and n=27, respectively).

When laboratory tests at the time of diagnosis were compared in patients according to their reassessed diagnosis (PSC=12 and PSC/AIH=13), the IgG level and ANA/SMA positivity were still statistically higher in the PSC/AIH group ($p=0.0001$ and $p=<0.0001$) and no other differences between groups were found.

The level of transaminases and GGT improved significantly during the follow-up period both in PSC and in PSC/AIH groups (Figure 1 and Figure 2).

Clinical course and prognosis

Long-term clinical outcome is shown in Figure 3. The median follow-up was 8 years (range: 2-20). At the last follow-up all the patients were alive. No cholangiocarcinoma or colorectal cancer was diagnosed during the follow-up period. At the end of the study, 30 patients

(85.7%) were still non-transplanted but three (10%) had cirrhosis, of which two had already at the time of diagnosis, one PSC patient was listed for LT, and one with PSC/AIH and progression of PSC underwent evaluation for LT. Twenty-eight out of 35 patients (80%) were on UDCA (at daily dosage of 450 to 1500 mg). Twelve patients (40%) were treated with immunosuppressant therapy: 3 PSC patients (2 with an associated IBD) and 9 PSC/AIH patients (6 with an associated IBD). Three PSC patients (one of them reassigned to the PSC/AIH) were without treatment.

During the follow-up, 4 patients were transplanted for PSC or PSC/AIH (11.4%) - 2 PSC and 2 PSC/AIH (1 PSC and 3 PSC/AIH after the reassessment) - after a median time of 7.5 years (range: 3-12) from diagnosis. Indications for LT were one each cirrhosis and refractory jaundice; cirrhosis (already present at the time of diagnosis) associated with repeatedly documented severe biliary dysplasia and aneuploidy; repeatedly documented severe biliary dysplasia and aneuploidy; and constant elevation of Ca19-9 and Ca12-5. One 9 year-old girl with PSC and cirrhosis at the time of diagnosis was transplanted one year later because of Budd-Chiari syndrome and thus she was not included in the survival analysis as it was not possible to establish whether or not cirrhosis was related to PSC. Kaplan-Meier curves of patient graft free survival for the PSC and the PSC/AIH patients are shown in Figure 4. No difference between the two groups of patients was seen ($p=0.9553$), even when patients were reassigned according to their re-reviewed diagnosis ($p=0.3716$). At the end of follow-up, all transplanted patients were alive and no patient had recurrence of disease in the graft.

DISCUSSION

Statement of principal findings. To our knowledge, this is the longest follow-up study in Europe investigating the clinical course and prognosis of paediatric onset PSC and PSC/AIH documented systematically with ERC imaging with brush cytology. To this respect, a better definition of the natural history of these diseases is pivotal as their prevalence has markedly increase during the last years, especially in Scandinavia, in line with the increasing incidence of IBD (116). The main findings of this study on the clinical course of the PSC in paediatric patients are: 1) the PSC/AIH is easily associated in children with PSC; 2) the concomitant AIH did not seem to affect the clinical course of PSC; 3) both the diseases may have a high risk for cirrhosis already at the time of diagnosis implicating the importance of more early diagnosis of the disease; and 4) both the diseases seem to have a good prognosis.

PSC/AIH overlap syndrome. In this series, PSC/AIH occurred in 37.1% of the patients and the rate increased up to 52%, when the diagnosis was reassessed (in those with available data) by using the simplified score for AIH, recently validated in children (114). This rate is in line with 50% reported by Gregorio et al (2) and lower compared with 25-35% found in other series (4, 6). This finding, however, confirms that PSC presents frequently with autoimmune features in children (2). Still, the high number of patients with PSC/AIH misdiagnosed at the time of diagnosis suggests a certain lack of awareness among clinicians and pathologists about these liver diseases (e.g., in the interpretation of liver biopsy) and it highlights the importance to address patients with a suspicion of PSC and PSC/AIH to a centre experienced in their management. The simplified score for AIH was demonstrated to be an easy and accurate method for the diagnosis of AIH both in adults (117) and in children (114). However, the score has been validated in a small sample of paediatric patients recruited from a single tertiary care hospital (114), which might lead to an overestimation of the performance

of the test. New studies are warranted in the future to confirm the accuracy and reliability of this score in children.

Presentation at the time of diagnosis. Children with PSC had a more frequently associated IBD, whereas those with PSC/AIH presented higher IgG level or circulating ANA/SMA. Otherwise, no other difference in clinical presentation at the time of diagnosis was found. Both of the diseases affected mostly boys with an onset in the second decade of life, as previously reported in the literature (4, 6, 45). Similar to two other large series (4, 6), most of the children (74%) had an associated IBD, UC being the most common (68%). Still, children with PSC suffered from IBD more frequently compared with their peers affected by PSC/AIH (91% vs. 46%, respectively). Recently, Deneau et al reported a comparable result in a paediatric cohort of PSC and PSC/AIH patients in Utah (U.S.A.), although the rate of IBD in the PSC/AIH group was as high as 75% (5). Gregorio et al found that 44% of PSC/AIH children had an IBD (2). So far, all studies have shown that PSC and IBD do not have a common genetic background (37, 39) supporting the paradigm of a unique phenotype PSC-IBD (27). Whether PSC/AIH-IBD and PSC-IBD share a common genetic link is difficult to ascertain because of the rarity of these conditions.

Because of the retrospective design of the study, we grouped the patients' symptoms and signs into four classes in order to better define the clinical presentation of PSC and PSC/AIH in children. The asymptomatic elevation of liver enzymes was the most common presentation (62.8%), as previously reported in the literature (45). Transaminases and GGT were elevated in most of the children and their level did not differ between the PSC and PSC/AIH group. The result was confirmed after the reassignment of the patients according to their re-reviewed diagnosis. Adults with PSC usually present cholestasis because the inflammation predominantly involves bile ducts (47, 48). In children, the inflammation seems to also affect the hepatocellular compartment. A higher level of transaminases and/or GGT in children with

PSC/AIH, compared to those with PSC, was reported in two studies (4, 5) but not in others (6). Interestingly, 35% of the children with PSC presented normal total ALP or bilirubin (73%) supporting the idea that ALP and bilirubin are unreliable markers of PSC. In adults, bilirubin is normal in up to 70% of the patients at the time of diagnosis (47). ALP was in normal range in 19% and 25% of PSC children in two other large series (4, 6) in contrast to adults that comprised 3% of the patients (118). In children, the hepatic isoenzyme of ALP might be a more accurate marker of PSC as bone maturation contributes to the total level of ALP (6). IgG level was higher and circulating ANA/SMA more common among PSC/AIH children confirming the florid autoimmune pattern seen in this disease subgroup of patients (2, 4-6). ANCA was not a specific marker to distinguish PSC and PSC/AIH.

In the current series, most of the patients (71.4%) had only intra-hepatic involvement and no patients presented isolated extra-hepatic disease. Feldstein et al found only intra-hepatic PSC in about 40% and only extra-hepatic in 2% of the children (6). Isolated extra-hepatic involvement is also less common in adults (55). In this study all the patients have been followed-up with ERC, which at our hospital is still considered the gold standard for the diagnosis of PSC. In the last years, MRI was reported as an accurate, safe, and cost-effective tool in the diagnosis of PSC although false negative and/or false positive cases do occur (64). ERC is an invasive procedure associated with complications such as pancreatitis, bleeding, perforation, and cholangitis (119-121), especially after papillotomy (122). However, the complications rate might be low in a high volume centre (123). In HUCH, over 1200 ERCP per year are performed and in PSC patients only a short papillotomy is usually done. The complication rate in PSC patients is low. In recent series of our unit consisting of 441 patients with PSC undergoing ERC, 7% had pancreatitis, of which two patients required hospitalization for two weeks and six patients developed cholangitis (1.6%) (57).

Clinical course and prognosis. All the patients were still alive in the last follow-up. Although in one study, the treatment with UDCA and immunosuppressant did not seem to have an impact on survival in patients with a paediatric onset PSC and PSC/AIH (6), most of our children received UDCA and most of those with PSC/AIH also immunosuppression.

Cirrhosis was present in two children each with PSC (9%) and with PSC/AIH (16%) at the time of diagnosis; two of them were transplanted. Miloh et al reported that 9% of the PSC children had cirrhosis at disease onset, and 56% of those affected by PSC/AIH had severe fibrosis or cirrhosis (4). Similarly, Feldstein et al reported severe fibrosis and cirrhosis in 54% of the PSC children at the time of diagnosis (6). Overall, LT was performed in approximately 11% of the patients after a median time of 7.5 years from the diagnosis of the liver disease. Survival free of LT was similar between PSC and PSC/AIH patients. Interestingly, in this study the rate of transplanted patients was lower compared to other studies with a similar follow-up, also showing a shorter time free of LT (4-6). In two single-centre series (4, 6) children with PSC or PSC/AIH required LT in 19% and 21% of cases after a mean and median time of 7 and 12.5 years, respectively. In a recent multi-centre study (5), 17% of the PSC-PSC/AIH children underwent LT. So far, none of our patients have showed recurrence of PSC and or AIH in the graft, although reported both in adults (106) and in children (6).

We did not identify cases of cholangiocarcinoma after a median follow-up of 8 years. In adults with PSC the lifetime risk of cholangiocarcinoma has been reported as high as 5-10% (73). In two single-centre paediatric studies cholangiocarcinoma was not identified after a mean and median follow-up of 6.6 and 6.3 years, respectively (4, 6). Conversely, in one population-based study cholangiocarcinoma was reported in approximately 7% of paediatric cases (5). We speculate that the follow-up protocol performed in our centre - which is based on endoscopic (i.e., ERC), radiologic (i.e., MRI/CT) and pathologic (i.e., cytology and flow-cytometry) findings – might help to select patients for optimal timing for LT and to prevent

complications. Interestingly, recurrent severe biliary dysplasia with aneuploidy on cytology samples and persistent elevation of neoplastic markers in one of each patient indicated LT, which probably have prevented the occurrence of malignancy. However, American (8) and European (9) guidelines recommend that ERC with brush cytology and/or fluorescence in situ hybridization (FISH) should not be considered as an evidence-based screening method for cholangiocarcinoma in PSC patients. Recently, Barr Fritcher et al reported that FISH might detect patients with polysomic cells in brushing samples before other techniques, identifying those having a higher risk of cholangiocarcinoma (124). New prospective multi-centre long-follow-up studies are warranted in order to establish the best diagnostic approach in these patients.

Strengths and weakness of this study. This study may be affected by a detection bias (i.e., the number of patients missed) as well as by a referral bias (i.e., diseases severity). We consider, however, the impact of these biases to be low as in Finland most paediatric patients with a suspicion of PSC are referred from all over the country to HUCH for the diagnosis and the follow-up. The strength of this study is the documentation of PSC diagnosis with ERC done by balloon occlusion technique to ensure visualisation of early intrahepatic changes. Moreover, the patient population was followed up by ERC and brush cytology. Conversely, the diagnosis of overlap syndrome could be reassessed in not more than 75% of the patients because of retrospective design of this study (i.e., missing samples) leading to a minor misclassification bias. However, the statistical analysis, comparing the two diagnostic groups, was performed by assigning the patients both to their original diagnosis and to their re-reviewed diagnosis. Finally, this study presents the longest follow-up of paediatric PSC patients reported in Europe.

CONCLUSIONS

In conclusion, the differential diagnosis between PSC and PSC/AIH might be challenging and PSC/AIH may be easily detected in PSC children. PSC and PSC/AIH may present with cirrhosis already at the time of diagnosis highlighting the importance of an earlier diagnosis of the disease. The overlap with AIH did not seem to affect the clinical course of PSC, but a strict follow-up in a centre with experience in the management of these diseases may have an impact on the prognosis.

CURRENT AND FUTURE STUDIES

A new prospective multicentre study on clinical course and prognosis of PSC in children is warranted in order to confirm the results of these few single centre series presented in the literature.

Moreover, many points remain challenging in paediatric onset PSC. Thus, I participated to the following studies during my working time in Finland:

1) one study aimed at investigating environmental risk factors in paediatric onset PSC and PSC/AIH overlap syndrome. The study is concluded and it shows that having a cat or having a dog in childhood may be a risk factor for the development of PSC and PSC/AIH overlap syndrome. This result may guide future preventive interventions for these diseases. The paper is under evaluation for publication.

2) one study including both adults and children aimed at evaluating the significance of early ERC and brush cytology to screen for PSC and biliary dysplasia in a large unselected PSC patient population coming to their first ERCP, to find factors associated with the development of biliary neoplasia and to study the relevance of brush cytology regarding the outcome. The study is concluded and showed that about 7% of the patients have high-grade dysplasia or cholangiocarcinoma at the time of their first ERC. Advanced PSC and elevated AST, ALT and GGT correlate to the development of biliary neoplasia. ERC with brush cytology may help to identify patients who need follow-up and eventually liver transplantation. The paper is under evaluation for publication.

3) one study aimed at reporting the role of MRCP in the diagnosis and follow-up of PSC patients comparing with ERC. This study will include both children and adults. The protocol is ready and it will start in January 2015.

TABLES AND FIGURES

Table 1. Modified Amsterdam PSC Score for primary sclerosing cholangitis

Score	Intra-hepatic system
0	No visible abnormalities
1	Ductural irregularities
2	Multiple calibre change; minimal dilatation
3	Multiple strictures; saccular dilations and decreased arborisation
4	Only central branches filled despite adequate filling pressure; severe pruning
	Extra-hepatic system
0	No visible abnormalities
1	Slight irregularities of duct contour; no strictures
2	Segmental strictures
3	Strictures of almost entire length of the duct
4	Extremely irregular margins; diverticulum-like outpunching

Table 2. Background characteristics and clinical presentation at the time of diagnosis of PSC or PSC/AIH in paediatric patients

	PSC (n=22)	PSC/AIH (n=13)	p
Median age and range (years)	15 (5-19)	15 (8-17)	0.8719
Male, # (%)	15/22 (68.2)	7/13 (53.8)	0.4803
Signs and Symptoms, # (%)			
• Insidious onset	13/22 (59.1)	8/13 (61.5)	1.0000
• Acute hepatitis	8/22 (36.4)	3/13 (23.1)	0.4776
• Acute liver failure	0/22 (0)	0/13 (0)	1.0000
• Complications of cirrhosis	1/22 (4.5)	2/13 (15.4)	0.5412
Associated IBD, # (%)	20/22 (90.9)	6/13 (46.1)	0.0060*
• UC	18/22 (81.8)	6/13 (46.1)	0.0571
• CD	2/22 (9.1)	0/13 (0)	0.5193
• Unclassified IBD	0/22 (0)	0/13 (0)	1.0000
Associated autoimmune disorders, # (%)	3/22 (13.6)	3/13 (23.1)	0.6485
Cirrhosis, # (%)	2/22 (9.1)	2/13 (15.8)	0.6176

PSC: primary sclerosing cholangitis; PSC/AIH: primary sclerosing cholangitis/autoimmune hepatitis overlap syndrome; IBD: inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease. Fisher's Exact Test for categorical variables and Mann-Whitney Test for continuous variables. *p significant when < 0.05.

Table 3. Histologic features on the liver biopsy at the time of diagnosis of PSC and PSC/AIH in paediatric patients after the reassessment of the disease.

	PSC n=12	PSC/AIH n=13
Grading (inflammation) # (%)		
0	9/12 (75)	2/13 (15)
1	3/12 (25)	2/13 (15)
2	0/12 (0)	4/13 (31)
3	0/12 (0)	5/13 (39)
Staging (fibrosis) # (%)		
0	4/12 (33)	3/13 (23)
1	3/12 (25)	3/13 (23)
2	2/12 (17)	3/13 (23)
3	2/12 (17)	2/13 (15)
4	1/12 (8)	2/13 (15)
PSC changes # (%)		
0	6/12 (50)	9/13 (69)
1	1/12 (8)	1/13 (8)
2	3/12 (25)	1/13 (8)
3	2/12 (17)	2/13 (15)
Interface hepatitis # (%)		
0	9/12 (75)	0/13 (0)
1	3/12 (25)	4/13 (31)
2	0/12 (0)	5/13 (38)
3	0/12 (0)	4/13 (31)

PSC: primary sclerosing cholangitis; PSC/AIH: primary sclerosing cholangitis/autoimmune hepatitis overlap syndrome.

Table 4. The biochemical and serological parameters at the time of diagnosis of PSC or PSC/AIH in paediatric patients

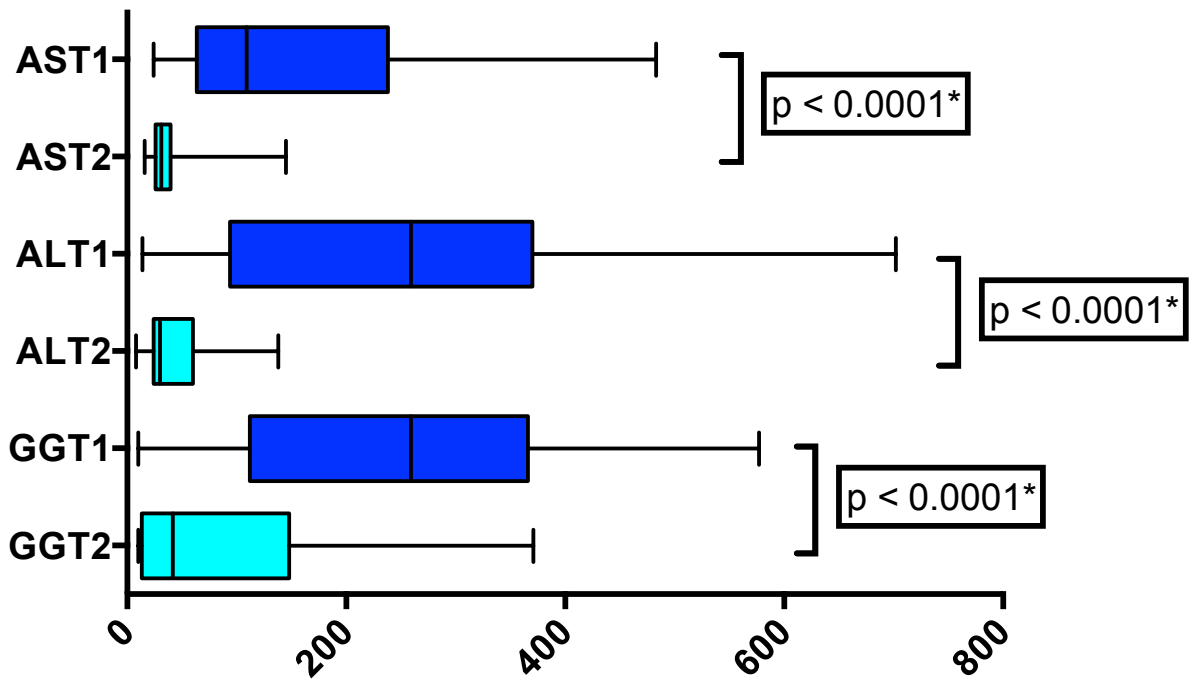
	PSC n=22	PSC/AIH n=13	p
Biochemical parameters			
• AST median (range) IU/L	114 (24-483) n=22§	219 (30-1385) n=12§	0.1335
• ALT median (range) IU/L	253 (14-702) n=22§	413 (88-2276) n=12§	0.0999
• GGT median (range) IU/L	257 (10-577) n=22§	178 (16-1089) n=12§	0.8137
• ALP °	14/22 (63.6) n=22§	8/12 (66.7) n=12§	1.0000
• Bilirubin median (range) mcmol/L	10 (5-45) n=22§	18.5 (6-169) n=12§	0.0430*
• Albumin median (range) g/L	41.4 (29-46) n=22§	35.7 (28.7-43) n=12§	0.0110*
• INR median (range)	1.1 (1.0-1.3) n=21§	1.1 (0.9-1.6) n=11§	0.7051
Serological parameters			
• IgG median (range) g/L	18.3 (9.1-27.6) n=22§	22.8 (9.2-88.8) n=11§	0.0352*
• IgM median (range) g/L	1.2 (0.6-2.6) n=22§	1.4 (0.4-2.0) n=10§	0.8338
• ANA and/or SMA (%)	5/22 (22.7) n=22§	8/12 (66.7) n=12§	0.0248*

• ANCA (%)	8/14 (57.1) n=14§	7/9 (77.8) n=9§	0.3998
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PSC: primary sclerosing cholangitis; PSC/AIH: primary sclerosing cholangitis/autoimmune hepatitis overlap syndrome; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyltranspeptidase; ALP: alkaline phosphatase; INR: international normalized ratio; IgM: immunoglobulin M; IgG: immunoglobulin G; ANA: antinuclear antibody; SMA: anti-smooth muscle antibody; anti-LKM1: antibody to the liver/kidney microsome type 1; pANCA: perinuclear antineutrophil cytoplasmic antibody.

§ number of patients tested. Continuous variables are expressed as median and range (in brackets), categorical variables like number and percentage (in brackets). °Rate of patient with ALP over the upper limit. Fisher's Exact Test for categorical variables and Mann-Whitney Test for continuous variables. *p significant when < 0.05.

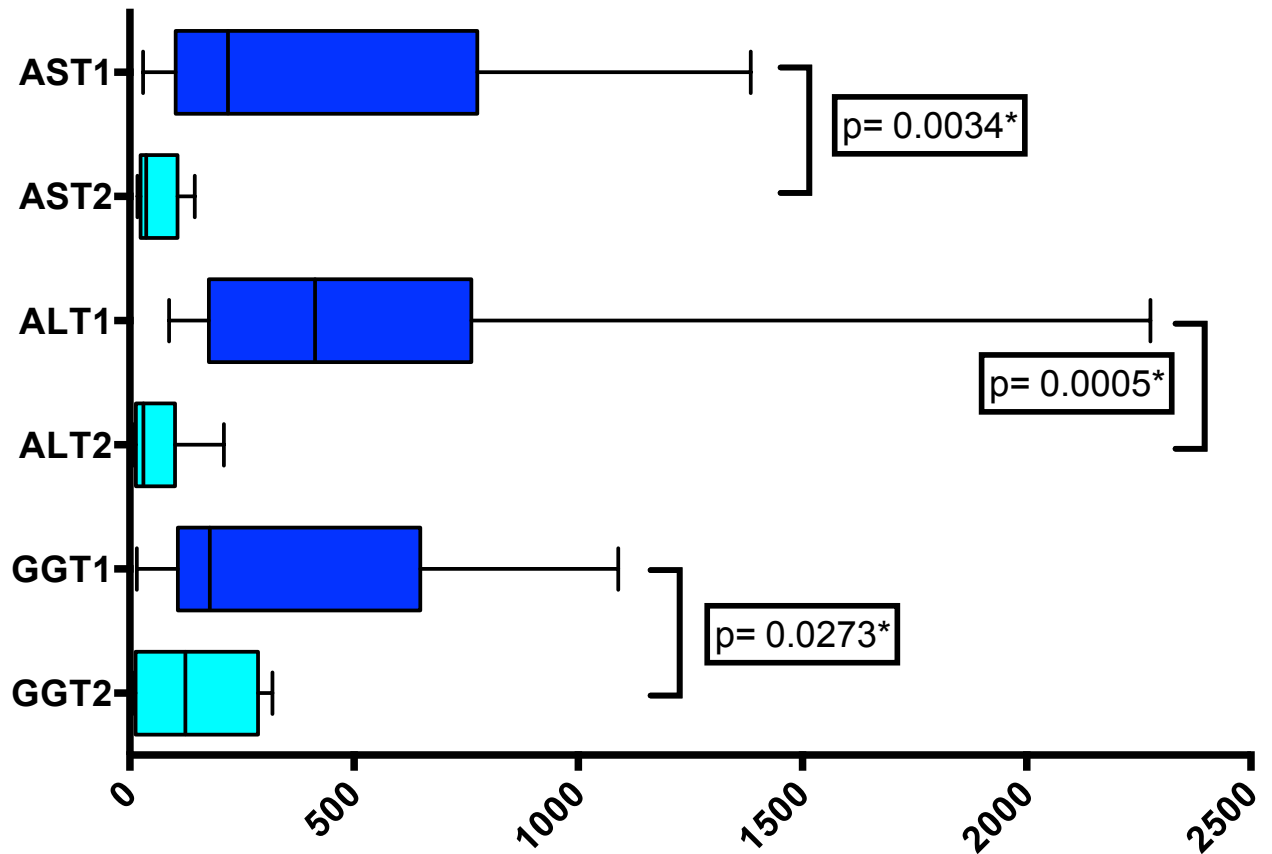
Figure 1. Transaminase and GGT level in paediatric onset PSC patients at the time of diagnosis (1) and at the last follow-up (2)



PSC: primary sclerosing cholangitis; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyltranspeptidase.

On y-axis liver tests at the time of diagnosis (1) and in the last follow-up (2) are reported. On x-axis the median value and range of liver tests expressed in IU/L are reported. Liver tests at the time of diagnosis and in the last follow-up were compared by Wilcoxon Test. *p significant when < 0.05.

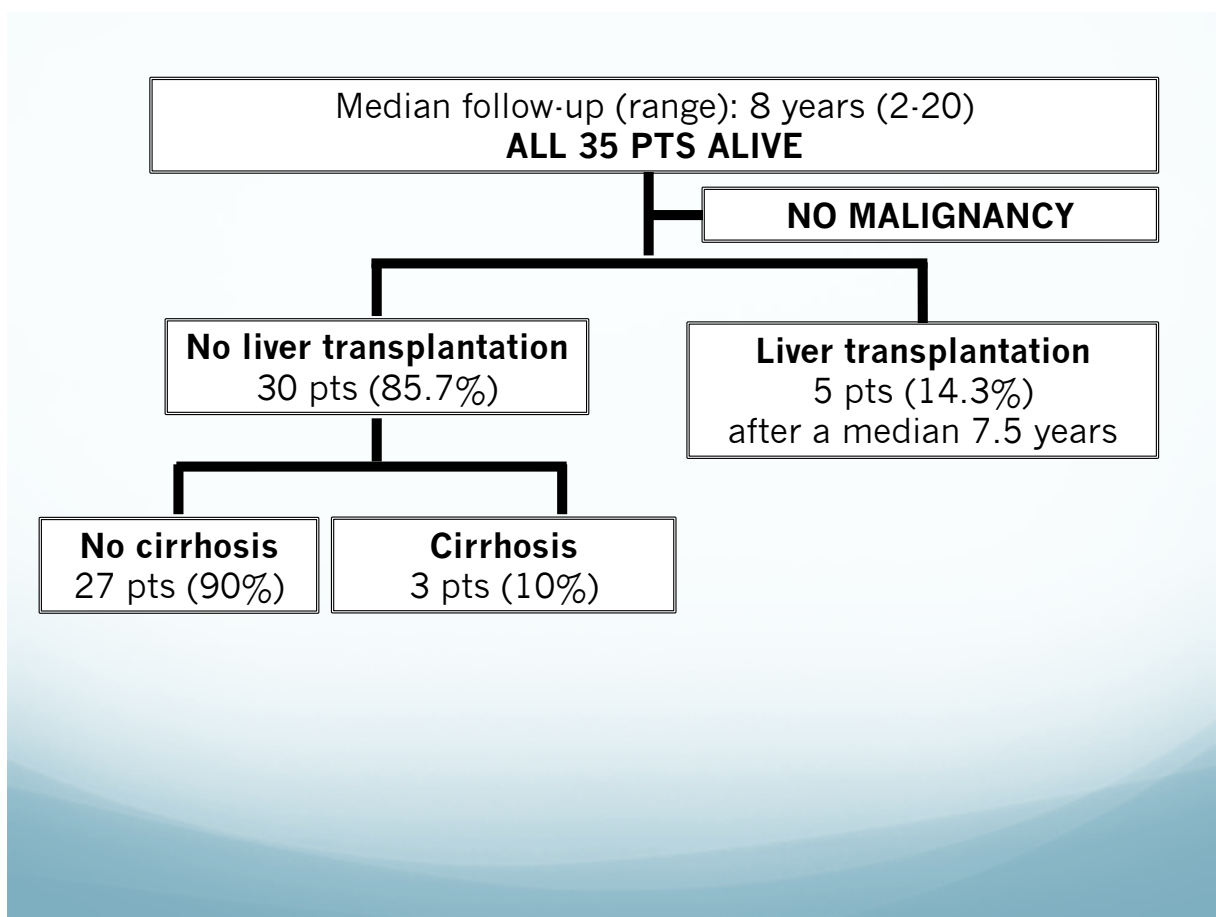
Figure 2. Transaminase and GGT level in PSC/AIH patients with paediatric onset at the time of diagnosis (1) and in the last follow-up (2)



PSC/AIH: primary sclerosing cholangitis/autoimmune hepatitis overlap syndrome; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyltranspeptidase.

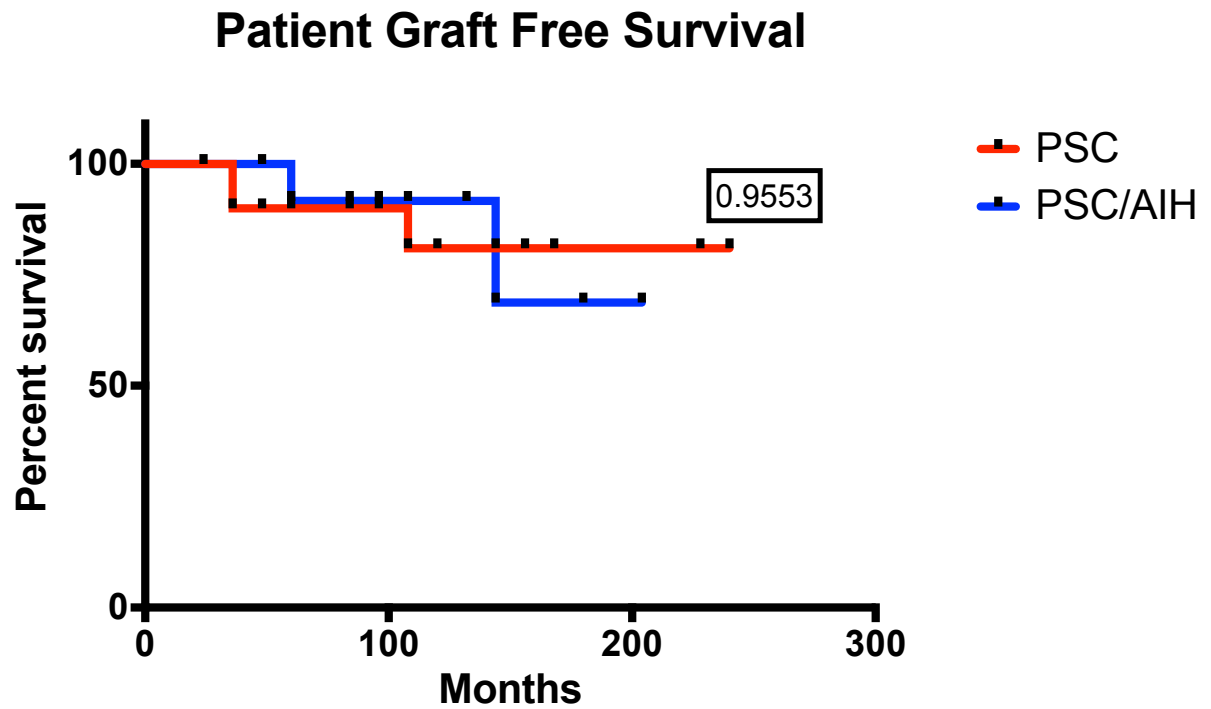
On y-axis liver tests at the time of diagnosis (1) and in the last follow-up (2) are reported. On x-axis the median value and range of liver tests expressed in IU/L are reported. Liver tests at the time of diagnosis and in the last follow-up were compared by Wilcoxon Test. *p significant when < 0.05.

Figure 3. Long-term outcome of PSC and PSC/AIH patients



PSC: primary sclerosing cholangitis; PSC/AIH: primary sclerosing cholangitis/autoimmune hepatitis overlap syndrome

Figure 4. Kaplan-Meier curves showing transplant free survival in patients with PSC and PSC/AIH.



PSC: primary sclerosing cholangitis; PSC/AIH: primary sclerosing cholangitis/autoimmune hepatitis overlap syndrome.

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