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From the Department of Medicine, University of Umeå, Umeå, Sweden

Primary Biliary Cirrhosis

An epidemiological and clinical study based on patients from Northern Sweden

by

PER UDDENFELDT

Umeå 1990

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An epidemiological and clinical study based on patients from Northern Sweden

AKADEMISK AVHANDLING
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av

Per Uddenfeldt

Umeå 1990

ABSTRACT

Uddenfeldt Per. 1990. Primary Biliary Cirrhosis. An epidemiological and clinical study based on patients from Northern Sweden.

From the Department of Medicine, Section for Gastroenterology, University of Umeå, S-901 85 Umeå, Sweden.

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Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease, which primarily affects middle-aged women. The liver histology is characterized by inflammation and destruction of the intrahepatic bile ducts as well as a high frequency of granuloma. Although the etiology is unknown, the occurrence of associated multiorganic abnormalities such as Sjögren's syndrome, scleroderma, rheumatic disorders and thyroid gland diseases have been cited as evidence favouring an autoimmune background.

Addison and Gull in 1851 described the first patient with jaundice and xanthomatosis. PBC was first mentioned in 1876 as an entity by Hanot. PBC was considered to be a rare disease until in 1973 Sherlock and Scheuer described 100 patients. Since then a greater awareness of the disease combined with a wider use of laboratory screening methods has led to the discovery of an increasing number of patients with PBC.

In an epidemiological investigation of PBC in the northern part of Sweden a point prevalence of 151 per 10^6 was found, which is the highest so far reported, and the mean annual incidence amounted to 13.3 per 10^6. Asymptomatic PBC was present in more than one third of the patients which is consistent with the finding in other epidemiological investigations and is supposed to explain the higher prevalence of PBC and the better prognosis. Nevertheless 25 patients died during the study period, 14 as a direct consequence of the liver disease. Chronic intrahepatic cholestasis has been reported in sarcoidosis and, moreover, a high frequency of liver granuloma is found. The implication of the present study is that a negative Kveim test in combination with positive mitochondrial antibodies is accurate in differentiating PBC from sarcoidosis. Multisystem involvement is frequently observed in PBC and the present study confirms this. In the prospective investigation of 26 PBC patients 50 % had arthropathy considered to be associated with PBC. Rheumatoid arthritis was found in 5 patients, who all had symptoms of liver disease in addition. Lung function impairment was present in 56% (1 asymptomatic PBC). Most commonly a reduced diffusion capacity was found (36%). Bronchial asthma was present in three patients, and severe lung emphysema in one. Features of Sjögren´s syndrome was found in 73% (3 asymptomatic PBC). In 6 patients keratoconjunctivitis sicca (KCS) was evident with the rose bengal test demonstrating corneal staining and a Schirmer test of less than 5 mm. Radiological findings of sialectasia were demonstrated in 6 patients, of whom 5 had KCS as well. The ultimate treatment in PBC is liver transplantation and to calculate the need for that, good epidemiological surveys are needed, and also indicators of hepatocellular function. The present investigation indicates that determination of the von Willebrand factor could be used for this purpose.

Key-words: Primary biliary cirrhosis, incidence, prevalence, disease course, Kveim test, sarcoidosis, rheumatic disorders, lung function, diffusing capacity, Sjögren syndrome, von Willebrand factor.

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PREFACE

The present thesis is based on the following original papers, which will be referred to in the text by their Roman numerals.

I. Danielsson Å, Boquist L, Uddenfeldt P.
Epidemiology of primary biliary cirrhosis in a defined rural population in the northern part of Sweden.
Hepatology 1990; 11:458-464.

II. Eklund A, Stjernberg N, Uddenfeldt P, Danielsson Å.
Kveim test and mitochondrial antibodies in the differential diagnosis between primary biliary cirrhosis and sarcoidosis.
Sarcoidosis 1985; 2: 38-41.

III. Uddenfeldt P, Danielsson Å.
Evaluation of rheumatic disorders in patients with primary biliary cirrhosis.
Ann. Clin. Res. 1986; 18: 148-53.

IV. Uddenfeldt P, Bjerle P, Danielsson Å, Nyström L, Stjernberg N.
Lung function abnormalities in patients with primary biliary cirrhosis.
Acta Med. Scand. 1988; 223: 549-55.

V. Uddenfeldt P, Danielsson Å, Forssell Å, Holm M, Östberg Y.
Features of Sjögren's syndrome in patients with primary biliary cirrhosis.
In manuscript.

VI. Danielsson Å, Nilsson T K, Uddenfeldt P.
Alterations in C1 Inhibitor and clotting factor concentrations in primary biliary cirrhosis and other chronic liver diseases.
Scand. J. Gastroenterol. 1990; 25:149-154.

ABBREVIATIONS

ALAT = serum alanine aminotransferase
ALP = serum alkaline phosphatase
AMA = antimitochondrial antibody
ANA = antinuclear antibody
ASAT = serum aspartate aminotransferase
DLCO = diffusing capacity of carbon monoxide
ERCP = endoscopic retrograde cholangiography and pancreatography
KCS = keratoconjunctivitis sicca
PBC = primary biliary cirrhosis
RA = rheumatoid arthritis
SS = Sjögren´s syndrome

BACKGROUND

Primary biliary cirrhosis (PBC) is a granulomatous liver disease characterized by a chronic non-suppurative destructive cholangitis. It affects primarily middle-aged and elderly women for the reasons unknown. PBC was regarded earlier as a rare disease but that standpoint has now been reconsidered in the light of an increasing number of reported cases of PBC, especially in the asymptomatic stage. The etiology is thought to be of autoimmune origin although so far no proof has been conclusive.

In 1851 Addison and Gull described the first patient with combined jaundice and xanthomatosis, and later in 1876 PBC was mentioned as an entity by Hanot. In 1950 Ahrens reviewed the literature on PBC and in its modern aspect PBC is based primarily on Sherlock and Scheuer's description (1973) of 100 patients with the condition. The diagnosis is based on a combination of clinical picture, laboratory findings and liver biopsy. Since then, a greater awareness of the disease in combination with a more widespread use of laboratory screening tests had led to the diagnosis of an increasing number of patients with PBC.

Symptoms of PBC can either be directly related to the liver disease or to conditions known to be associated with PBC. Included in the context of symptomatic PBC are: jaundice, pruritus, hyperpigmentation, xanthoma, xanthelasmata, and/or hepato-splenomegaly. When these signs and symptoms are missing the patient is classified as asymptomatic, although symptoms from associated diseases such as Sjögren's syndrome or rheumatoid arthritis may be present.

Laboratory abnormalities can either be biochemical or serological. Liver function tests most typically show a cholestatic pattern with ALP more than twice the normal value, while ASAT and ALAT are only slightly

increased. High serum bilirubin value is an ominous sign occurring late in the course of the disease. IgM is often increased, and AMA is abnormal in 90 - 100 % of the patients. In liver biopsy specimens four stages can be separated but only in stage 4 is there cirrhosis. The presence of florid duct lesions, i.e. stage 1, is diagnostic for PBC, whereas if these lesions are missing, the biopsy only may be compatible with PBC, more convincingly so if granuloma are found.

ETIOLOGY

While the etiology of PBC is still unknown (Jones 1983; Epstein 1985) many authors are convinced of its autoimmune origin (James et al 1983). Conclusive evidence, however, is still lacking. One reason for the hypothesis is the close association of PBC with rheumatoid arthritis (RA), keratoconjunctivitis sicca (KCS), Hashimoto´s disease and Sjögren´s syndrome (SS) (Golding et al 1973). Several immunological abnormalities are found in PBC, such as the presence of circulating serum antibodies and immune complexes, the formation of granuloma, complement activation and impaired delayed hypersensitivity (Sherlock 1982). Moreover, there is an increased familial incidence of the disease and a marked female predominance (Sherlock & Scheuer 1973).

PBC has been reported from all over the world and in all races. However, previous studies on the epidemiology of PBC have shown varying prevalences. In Europe the point prevalence has been reported to be 23 per 10^6 population and the annual incidence 4 cases per 10^6, with marked variation between different countries (Triger 1984). In primary sclerosing cholangitis, there is a strong association between HLA-DRw52a, whereas no or moderate association with conventional autoimmune HLA-antigens has been found in PBC (Hamlyn et al 1974, Prochazha et al 1990). The expression of HLA antigen on biliary

epithelium may cause an immunological attack, and such a reaction may be due to a defect in the immune system or to the fact that HLA antigens are detected as foreign by the immune system perhaps induced by some extrinsic environmental factor. Virtually all PBC patients have a positive AMA test. Some studies have reported similarities between the AMA-antigen and parts of certain microorganisms, which thus might be one possible extrinsic factor involved.

In the city of Sheffield 90 % of the PBC patients came from an area that only contained 4% of the population (Triger 1980), and it has been suggested that the cause could be the single water reservoir for this area. However, so far no environmental causative factor has been isolated from the water supply.

ANTIMITOCHONDRIAL ANTIBODIES (AMA)

In an unselected population of adults, positive AMA, i.e. a titre of 1/25 or more, is found in about 0.3-0.6% (Triger et al 1982). Almost all PBC patients present a positive AMA, especially when using an undiluted serum for the analysis (Munoz et al 1981). There is no correlation between the AMA titre and the stage or activity of the disease. Although the sensitivity of the test is good the specificity is less reliable. AMA is found in about 10% of patients with autoimmune chronic active hepatitis (CAH) and in 5% with cryptogenic cirrhosis, as well as in some patients with drug-induced hepatitis (Klatskin & Kantor 1972). Moreover, mitochondrial antibodies have also been demonstrated in several non-hepatic diseases (Berg 1973) such as rheumatoid arthritis (2%), SLE (5%), Sjögren´s syndrome and scleroderma (8%). In polymyalgia rheumatica, about one third of the patients display positive AMA tests (Sattar et al 1984), which also is the case in secondary syphilis (Doniach et al 1970).

The AMA test is usually carried out by an indirect immunofluorescence technique using a polyvalent immunoglobulin conjugate with rat liver, kidney and stomach as substrates (Walker et al 1965). Other techniques are complement fixation test (Berg et al 1980), radioimmunoassay (Manns et al 1982), and enzyme-linked immunosorbent assay (ELISA) (Nagai et al 1983). These methods have revealed the existence of various subtypes of AMA. The M2 antibody is characteristic of PBC and directed against a component of the mitochondrial inner membrane. The M4 antibody probably has the antigen located at the outer mitochondrial membrane and occurs mostly in combination with M2. Recent immunological studies have suggested that the M2 antigen is identical with the E2 component of the mammalian pyruvate dehydrogenase complex (PDC), which consists of 4 components and that the majority of these antibodies are both of IgG and IgM type (Mutimer et al 1989).

HISTOLOGY

PBC is a cholestatic disease in which intrahepatic bile ducts of small calibre are destroyed as part of a granulomatous reaction. The histological classification is divided into four stages (Sherlock & Scheuer 1973). Cirrhosis is seen only in stage 4, and the name PBC may thus be misleading. Chronic non-suppurative destructive cholangitis has been suggested as a more appropriate name for the disease without success, however (Rubin et al 1965).

Stage 1 shows a florid, asymmetric destruction of the septal and interlobular bile ducts and what are typically surrounded by dense infiltrates of mononuclear cells, especially T lympocytes. Hepatic granuloma can be seen in all stages but are most common in stage 1. The presence of granuloma has been proposed to be of prognostic importance (Lee et al 1981). However, this observation has not be confirmed (Roll et al 1983).

In stage 2 there are more widespread lesions with a reduction of normal bile ducts and increased numbers of atypical, poorly formed bile ducts. Diffuse portal fibrosis is seen and as in stage 1 periportal cholestasis is conspicuous.

Stage 3 displays more progressive lesions with fibrous septa forming bridges.

Stage 4 represents the end stage with clear cirrhosis and may be difficult to distinguish from other types of cirrhosis.

The lesions may be patchy and all stages can be represented in the same liver and sometimes even in the same needle biopsy. Staging should be based on the most severe lesion present.

CLINCIAL PRESENTATION

Symptoms of PBC pruritus are jaundice, hyperpigmentation, xanthoma, xanthelasmata, and/or hepato-splenomegaly. In advanced PBC, complications to cirrhosis may occur, e.g. gastrointestinal bleeding, ascites, hepatic coma and death. If all of these signs and symptoms are missing the patient is classified as asymptomatic.

TREATMENT

There is no definite cure for PBC. Trials of corticosteroids, azathioprine, penicillamin, cyclosporin, clorambucil, cholchicine and ursodeoxycholic acid (UDCA) have not to date presented any convincing results that could form a basis for treatment in clincial practice. The ultimate therapy is liver transplantation, the results of which are improving with time. Pruritus, osteoporosis and hypercholesterolemia are treated by conventional means.

Corticosteroids

In autoimmune chronic active hepatitis corticosteroids have proved to be an effective treatment in reducing inflammation and preventing, or at least postponing the development of cirrhosis (Cook et al 1971). There are few trials of corticosteroid treatment in PBC. Seven patients were described by Howat et al (1966) to have benefitted from corticosteroids, but experienced a number of side effects, notably infections and osteoporosis. Since PBC, per se, has a high incidence of osteoporosis corticosteroids have been regarded as contraindicated (Sherlock & Scheuer 1973). However, in a recent pilot study (Mitchison et al 1989) it was demonstrated that prednisolone treatment in doses similar to those used in chronic active hepatitis appears to retard disease progression in PBC. Prednisolone was tolerated well, but even in the moderate doses used during a period of one year an accelerated loss of bone was detected.

Azathioprine

In two studies azathioprine has (Heathcote et al 1976; Crowe et al 1980b) been shown to have no or only little effect in PBC. However, one of the studies which was continued for a longer period of time suggests that survival may be prolonged (Christensen et al 1985). Only minor side effects were encountered. In this multinational double-blind trial 248 patients were included, and 127 received azathioprine. These patients were later shown to have a more advanced disease than those receiving a placebo. When corrected for this, azathioprine was found to induce a significantly prolonged survival time of almost two years. No other study has been carried out to confirm the results.

Penicillamin

Penicillamin is capable of removing copper from the liver and other tissues and is therefore the treatment of choice in Wilson´s disease. In PBC, the liver also displays an accumulation of copper secondary to the

cholestasis, so penicillamin could be a reasonable treatment although there are certain biochemical, histological and histochemical differences between PBC and Wilson´s disease (Epstein et al 1981a). In addition, penicillamin has immunosuppressive and antifibrotic properties. Despite several studies dealing with penicillamin treatment in PBC, no conclusive evidence of any positive effect has been presented, and the side effects are many (Bodenheimer et al 1985; Dickson et al 1985; Neuberger et al 1985; Matloff et al 1982). Nevertheless, Epstein et al (1981b) recommend the use of penicillamin in late stage 3 and stage 4.

Cyclosporin

Cyclosporin is an immunoregulatory drug and in PBC it has been shown to increase the proportion of T suppressor cells in relation to inducer cells in peripheral blood and to cause improvement in liver function tests, which may reflect a decreased inflammatory activity (Routhier et al 1980). However, the side effects of cyclosporin, especially the risk of renal damage, make the drug less attractive. Recently, treatment with a low-dose regimen if cyclosporin has produced promising results and only slight renal toxicity (Wiesner et al 1990).

Clorambucil

Clorambucil has been shown to improve liver function tests, but its toxicity seems to neutralize the positive effect. In one study including 13 patients on chlorambucil, 4 presented severe bone marrow suppression and the therapy could not be reinstituted (Hoofnagle et al 1986).

Cholchicine

Cholchicine, apart from causing diarrhoea, seems to be atoxic and some improvements of liver function tests in PBC have been reported, whereas no reduction in hepatic inflammation has been encountered (Kaplan et al 1986).

Ursodeoxycholic acid (UDCA)

Some of the hepatic lesions seen in chronic cholestasis could be a result of hepatocellular accumulation of toxic bile acids. Poupon et al (1985) showed promising results when they treated PBC patients with UDCA. Pruritus was significantly reduced and standard liver function tests improved in all patients. Patients who interrupted the intake of UDCA showed an impairment in liver function tests, which disappeared when therapy was restarted. A double-blind study later confirmed the beneficial effect of UDCA on liver function tests (Leuschner et al 1989) in 20 patients while only a slight improvement in histology, all in stages 1 or 2, was found. Whether UDCA can modify the progress of PBC remains to be proved.

Liver transplantation

During recent years successful liver transplantations have been carried out in cases of severe liver disease. In fact, patients with end stage PBC seem to be excellent candidates for liver grafting. Esquivel et al (1988) reported a 66% 5-year survival rate. All of these patients had advanced PBC and all but one were jaundiced. Most of the deaths occurred within the first 6 months after transplantation. All patients received cyclosporin and corticosteroids, but retransplantation was necessary in 25%. In the patients tested for AMA, the test was still positive after transplantation, but there was no evidence of a recurrence of PBC, such as was previously reported by Neuberger et al (1982). In a comparison between 161 transplanted PBC and a conservatively treated group Marcus et al (1989) conclude that liver transplantation is an efficient treatment for advanced PBC. The quality of life seems to be excellent with more than 90 % returning to work after a successful operation.

AIMS OF THE PRESENT STUDY

These were as follows:

* to study the epidemiology and clinical characteristics of patients with PBC in the northern part of Sweden.

* to study the differential diagnosis of PBC versus sarcoidosis.

* to study extrahepatic manifestations of PBC with special regard to:
  the occurrence and characteristics of joint symptoms,
  lung function impairment
  and occurence of Sjögren's syndrome.

* to study alterations in plasma proteins and clotting factor concentrations as prognostic factors in PBC.

PATIENTS AND METHODS

DIAGNOSTIC CRITERIA

The diagnosis of PBC was based on a combination of clinical signs, presence of increased ALP, IgM and AMA as well as a liver biopsy compatible with the criteria determined by Sherlock and Scheuer (1973). If no liver biopsy was available a positive titre of AMA ($\geq$1/25) combined with an increased ALP (>5.1 ukat/L) was a prerequisite. When appropriate, extrahepatic bile duct patency was established radio- or sonographically. Patients with jaundice, pruritus, hyperpigmentation, xanthoma, xanthelasmata, or hepato- and splenomegaly were classified as symptomatic.

STUDY POPULATIONS

The region served by the University Hospital of Umeå consists of a primary catchment area of 120,000 inhabitants and was at the time of the investigation the centre of specialized medical care for a further population of 450,000. The Northern Health Region had 10 hospitals in all for the population of 570,000.

Study I

In this study 111 patients with PBC were investigated of whom 96 were females. All known cases of PBC within the region were reported on request and registers in the departments of medicine, surgery and infectious diseases in the 10 hospitals within the region were searched for patients with cholestatic liver diseases.

All positive AMA tests at the Department of Clinical Bacteriology, Umeå and the National Bacteriology Laboratory (SBL), Stockholm were followed up during the period 1979-1981. On December 31, 1982 to our knowledge 86 patients with PBC were alive and made up the prevalence population.

Studies III, IV and V

These studies were based on 26 consecutive patients with PBC admitted to the Gastroenterological Unit at Umeå University Hospital during a 2-year period (1980-81). The mean age was 56 years (range 39-77), 24 were females and 7 were asymptomatic as regards the liver disease. For technical reasons a liver biopsy was not obtained from one patient and in another two there were contraindications performing the biopsy. In study IV one patient was excluded because of a postoperative pleura empyema which prevented her from performing the lung function tests.

Study II

Of the patients included in Studies III - V, 14 were subjected to a Kveim test. The mean age was 53 years (range 39-64) and 5 had asymptomatic PBC. This group was compared with a group of 38 patients with sarcoidosis.

Study VI

The study consisted of 46 patients of whom 27 had PBC. The PBC patients had a mean age of 57 years (range 39-78) and 14 had asymptomatic PBC. Eight patients had cholestatic liver disease of whom 3 had primary sclerosising cholangitis and 5 had idiopathic cholestasis with cirrhosis. The remaining 11 patients had cirrhosis without evidence of cholestasis: 7 alcoholic cirrhosis, 3 autoimmune chronic active hepatitis and 1 α-1-antitrypsin deficiency.

ANALYSES

Liver biopsies

Liver biopsies were performed ad modum Menghini and fixed in buffered formalin. Liver histology was examined by Professor Lennart Boquist, Department of Pathology, Umeå University Hospital and classified according to Sherlock and Scheuer (1973) into four stages: 1) florid duct lesion; 2) ductular proliferation; 3) fibrosis; and 4) cirrhosis.

Antimitochondrial antibodies (AMA)

The serum test for mitochondrial antibodies was performed by the immune fluorescence technique in serial dilution at the Department of Clinical Bacteriology of Umeå University Hospital or the National Bacteriological Laboratory (SBL), Stockholm. A titre of $\geq$ 1/25 was regarded as positive.

Routine analyses

Liver function tests, P-immunoglobulins (immune diffusion technique) and P-proteins were analysed at the Department of Clinical Chemistry and tissue typing for presence of HLA B27 antigen using specific antisera was carried out at the Blood Bank of the University Hospital of Umeå.

Clotting factors

C1-inhibitor activity was measured in citrated plasma with an assay based on the addition of an excess of purified C1s to the sample, and measuring the remaining C1s with the chromogenic substrate D-Val-Ser-Arg-p-nitroanilide (S-2314), as previously described (Wiman et al 1983). von Willebrand factor (vWF) was measured in citrated plasma with an enzyme-linked immunosorbent assay (ELISA) (Cejka 1982), using antibodies and horseradish peroxidase (HRP). Antithrombin (AT) was measured with a chromogenic substrate kit.

Kveim test

The Kveim suspension was prepared from a sarcoid spleen and 0.15 mL was injected subcutaneously into the thigh. The injection site was marked with India ink. After 6 weeks a punch biopsy was performed. The test was regarded as positive if there was microscopical focal aggregations of epitheloid cell (Williams et al 1976).

Pulmonary physiology

Lung volumes were measured with the closed circuit helium dilution method for functional residual capacity determination, and dynamic spirometry was assessed by means of the Bernstein spirometer (Berglund et al 1963). DLCO was recorded by the single breath technique described by Ogilve et al (1957).

Schirmer test

Five mm of a sterile standardized strip of blotting paper was folded at the end and inserted into the lower conjunctival fornix. After 5 minutes the strip was removed and the the length of the paper wetted was measured from the fold (abnormal < 5 mm).

Rose bengal test

One drop 1% rose bengal was installed in each conjunctival sac. After some blinks irrigation with physiological saline followed, the eye was photographed and considered normal when no staining was seen in either conjunctiva or cornea.

STATISTICAL METHODS

Student's t-test was applied to test differences between groups (**I, IV**).
To test differences between the means of more than two groups the Wilcoxon nonparametric test was used (**I, VI**).
Linear regression using the least square method was used to study the correlation between DLCO and maximal working capacity (**IV**).
Spearman´s rank correlation test was used to study relations between variables (**VI**).
Chi-2-test was used to study the dependence between variables based on a categorical scale (**IV**).

RESULTS AND DISCUSSION

ANTIMITOCHONDRIAL ANTIBODIES (AMA)

During the period 1979-1981 25,000 serum samples were analysed of which 144 were AMA positive at a titre of 1/25 or more. The medical notes on these patients were scrutinized. Of these 144 patients, 71 had medical data consistent with PBC, but only 3 were not previously known to us. The medical notes were unavailable for 8 patients. Of the remaining 65 patients with positive AMA with no signs of PBC, 63% had collagenous disease and/or musculoskeletal symptoms of which RA was the most common (16%) (Table I).

Table I: Diagnoses of patients with positive AMA with no evidence of PBC (n=65).

Biopsy verified liver diseases (12)
- CAH (3)
- Hepatocellular carcinoma (3)
- Hemochromatosis (1)
- Alcohol hepatitis (3)
- Steatosis (2)

Infectious diseases (5)
- Virosis NUD (3)
- Chronic pyelonephritis (1)
- Secondary lues (1)

Collagenous diseases (29)
- SLE (8)
- RA (11)
- Arthritis (4)
- Scleroderma (3)
- Polymyalgia rheumatica / temporal arteritis (3)

Musculoskeletal symptoms (12)
- Arhralgia (7)
- Tendinitis (3)
- Myopathia (2)

Miscellaneous (7)
- Ulcerative colitis (1)
- Thyreotoxicosis (4)
- Sarcoidosis (2)

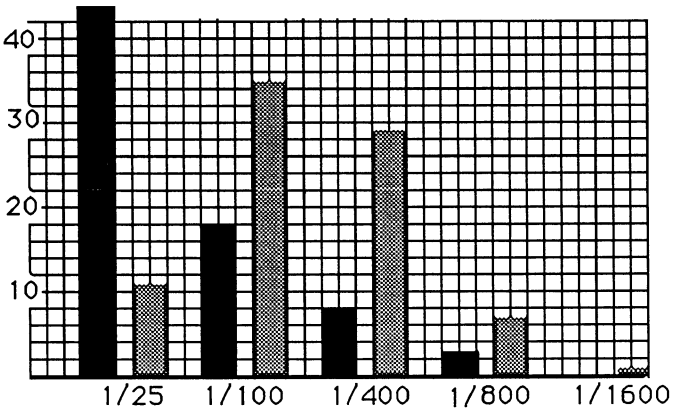

Fig. 1: AMA titres in patients with PBC (n=83) or other diseases (n=73)

Earlier studies have also found that about 50% of patients with positive AMA tests did not have PBC (Löfgren et al 1985). However, these patients represent a selected group of patients. The high frequency of musculoskeletal disorders may be explained by the clinical routine of carrying out ANA tests in these patients and that AMA and ANA are analysed together. In an adult healthy population the prevalence of positive AMA is about 0.3-0.6% (Triger et al 1982). Among these individuals, in spite of normal ALP, a liver biopsy may reveal a concealed PBC (Mitchison et al 1986). There are reports of positive AMA in autoimmune chronic active hepatitis, cryptogenic cirrhosis and collagenous diseases in agreement with the present results (Munoz et al 1981; Table I).

On December 31, 1982 there were 86 PBC patients alive, of whom 83 had a positive AMA. The titres of these patients were compared with the remaining patients without evidence of PBC and there is a tendency for the latter to have lower titres of AMA (Fig. 1), which confirm the results

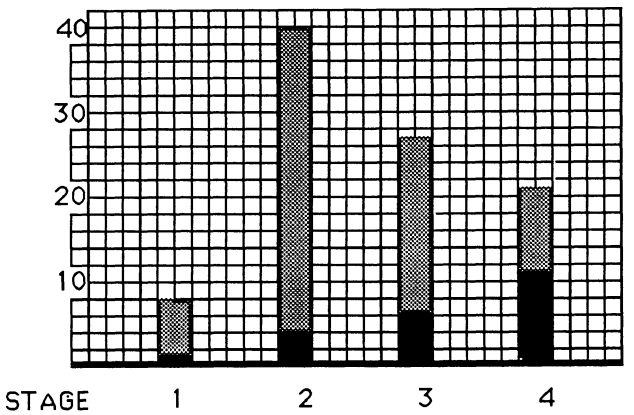

Fig. 2: Histological stage of liver biopsies in relation to outcome (n=96).
Alive on December 31, 1982
Dead

of Hasselström et al (1988). In patients with PBC there was no correlation between AMA titre and duration or severity of the disease. Therefore, when the diagnosis of PBC is established there is of no clinical value in a prospective check of the AMA-titre.

EPIDEMIOLOGI (I)

In total 111 patients with PBC (96 females and 15 males) were identified during a 10-year period (1973-1982) in the Northern Health Region in Sweden. This area is sparsely populated with a mean density of 3 inhabitants per km^2 and must be regarded as a rural area. Liver biopsies were available in 96 patients and stage 2 was most common among patients still alive at the end of 1982. During the study period 25 patients died (23 %) with stage 4 overrepresented (Fig. 2).

Incidence and prevalence

In 35 cases out of the 111 patients the diagnosis was established before 1972. The 76 patients with PBC diagnosed during the actual period gave a mean annual incidence of 13.3 per 10^6 (range 8.8 - 21.0) with some more cases especially asymptomatic ones discovered during the later period of the study (I). The incidence rate of PBC in Sweden (Eriksson & Lindgren 1984; Löfgren et al 1985) exceeds that found in other countries in Europe with the exception of an urban population in Newcastle, UK, where a similar incidence has also been recorded (Hamlyn et al 1983). As the present study covers a mainly rural area, it does not lend support to the theory that causative agents are present in urban environments.

On December 31, 1982, 86 patients were alive. That makes a point prevalence of 151 per 10^6 in Northern Sweden and it was significantly higher ($p<0.01$) in the most northerly county (BD) compared with the county in which the university hospital is located (AC), 186 vs 97 per 10^6 with the symptomatic cases also overrepresented (I). The prevalence of PBC encountered in the Northern part of Sweden is the highest so far reported. The prevalence of the disease seems to vary considerably among different countries (Table II). A higher prevalence has been found in industrial than in rural areas (Hamlyn et al 1983) and clustering has been found around certain water reservoirs (Triger 1980). In the present study the highest figures were obtained from the most northerly county, which is characterized by mining and the steel industry.

Epidemiological studies are influenced by an effective discovery and diagnosis of the cases. In this study, asymptomatic PBC with few or no clinical manifestations of the disease in particular could have been missed, and thus, the figures for incidence and prevalence may be an underestimate.

Table II: Summary of previous reports on the annual incidence and prevalence of PBC.

Place of study	Years	Mean annual Incidence per 10^6	Point Prevalence per 10^6	Type of Community	Population size 10^6	Number of Cases	Reference
Newcastle UK	1972-1979	10.0	144 (72)*	Urban	2.08	117	Hamlyn et al 1983
Malmö Sweden	1973-1982	13.7	92 (51)*	Urban	0.24	33	Eriksson & Lindgren 1984
Sheffield UK	1977-1979	5.8	54 (49)*	Urban	0.52	34	Triger 1980
Cities in Europe	1977-1981	4.0	23 (23)*	Urban	24.6	569	Triger 1984
Örebro Sweden	1976-1983	14.0	128 (64)*	Mixed	0.16	18	Löfgren et al 1985
Northern Sweden	1972-1982	13.3	151 (95)*	Rural	0.57	111	I

*Prevalence of symptomatic PBC calculated from the respective study (within brackets)

However, the combination of a national health system, a stable population, plus a well organized population register and hospital registration of diagnoses, minimizes the loss of patients with symptoms and an established diagnosis of asymptomatic PBC. The high incidence and prevalence figures for PBC in the Swedish studies could either reflect a real increased occurrence of the disease, or it could be a consequence of greater awareness of the disease and/or the efficient functioning of medical registers.

KVEIMS TEST (II)

Not only PBC but also other granulomatous diseases seem to occur more frequently in Sweden, especially in the Northern part. High incidences and prevalences of sarcoidosis (Stjernberg & Wiman 1972) and Crohn's disease (Nyhlin & Danielsson 1986) have been reported, which may suggest a common etiological factor for the development of

granulomatous diseases. Pulmonary dysfunction is common in patients with PBC (Rodriguez-Roisin et al 1981) with a pattern similar to those found in sarcoidosis (**IV**). Granuloma in the liver can be found not only in PBC but also in sarcoidosis which sometimes manifests itself as a chronic intrahepatic cholestasis (Rudzki et al 1975). Recent investigation of PBC with bronchoalveolar lavage has revealed a subclinical alveolar inflammation with T-lymphocytes and activated alveolar macrophages in a high proportion of patients with PBC mimicing sarcoid alveolitis (Wallaert et al 1986). These findings focus on the importance of differential diagnosis between PBC and sarcoidosis.

Stanley et al (1972) have suggested, on the basis of 2 cases, that the findings of a negative Kveim test in combination with a positive AMA is the the best way to differentiate PBC from hepatic sarcoidosis. In order to verify this suggestion we investigated 14 PBC and 38 patients with sarcoidosis. In the sarcoidosis group all had a negative AMA (<1/25) and 63% had a positive Kveim test. The Kveim test usually has a positive result in 74% in sarcoidosis (Turiaf et al 1980) and AMA in about 95% in PBC (Munoz et al 1981). None of the PBC patients had a positive Kveim test and all had a positive AMA. In this study sarcoidosis and PBC seem to be two distinct disorders.

EXTRAHEPATIC SYMPTOMS (III-V)

Extrahepatic symptoms seem to be common in patients with PBC (Golding et al 1973) and therefore 26 consecutive patients were investigated over a 2-year period. Seven patients were asymptomatic with respect to the liver disease and 42% of the patients had a liver histology compatible with stage II, 35% with stage III and 12% with stage IV, while none presented the features of stage I. Liver biopsy was not performed in 3 patients.

Rheumatic disorders (III)

Joint symptoms of various kinds were encountered in 18/26 (69%) of the patients with PBC. In five of these patients the musculoskeletal symptoms were considered to have a specific cause not directly related to the liver disease. In the remaining patients arthritis was found in 7 patients, while 6 had arthralgia a finding which is in agreement with some previous reports of joint symptoms and PBC (Child et al 1977; Clarke et al 1978). Of these 13 patients with arthropathy, 2 were asymptomatic and had no signs of arthritis.

All patients with arthritis (27%) complied with the American Rheumatism Association's definition of rheumatoid arthritis (Ropes et al 1958). According to these criteria 2 patients had possible RA whereas according to the new criteria (Arnett et al 1988) only the remaining 5 had RA. Nevertheless, the prevalence of 18% with classical (2 patients) and definite rheumatoid arthritis (3 patients) exceeds both that expected for an unselected population (Lawrence 1961), and that found previously in a study of patients with PBC (Sherlock & Scheuer 1973). Six patients (23%) had arthralgia with no evidence of arthritis at the time of the investigation, although the symptoms were similar to the group with arthritis, presenting bilateral polyarticular involvement of both small and large joints. All patients with arthritis or arthralgia developed their arthropathy after the onset of the liver disease, which relates the joint symptoms more closely to the liver disease. The mean age of the patients with arthropathy was lower than for those without, indicating that age has no direct influence on the arthropathy, which is in agreement with Willcox and Isselbacher (1961), who found arthropathy in 36% of young female patients with chronic liver disease.

The mechanism for the association between rheumatic symptoms and PBC is unknown. However, a high prevalence of autoimmune disorders in PBC has been reported (Culp et al 1982). Immunological disturbances

have also been suggested as a cause of arthritis in viral hepatitis (Alpert 1971) and autoimmune chronic active hepatitis (Wands 1975). Increased levels of circulating immune complexes have been recorded in PBC (Thomas 1978), which could explain tissue damages outside the liver, and Crowe et al (1980c) found circulating immune complexes in 62% of PBC patients. The corresponding figure in the present study (**III**) was 35% as demonstrated by the C1q binding technique. However, there was no correlation with the presence of arthropathy.

In one study of PBC chondrocalcinosis was found in 2 out of 13 patients (O´Connell & Marx 1978), which, however, was not the case in the present investigation. Cholestasis induces increased levels of plasma cholesterol and hypercholesterolaemia may thus be an etiological factor for joint symptoms in PBC. Radiologically arthritis caused by hypercholesterolemia may be indistinguishable from rheumatoid arthritis (Ansell & Bywater 1957). However, in the present study there was no correlation between serum cholesterol levels and joint symptoms. Osteopenia is a widely recognized complication of longlasting cholestatic liver diseases, notably PBC (Atkinson et al 1956), which to a certain extent is caused by steatorrhea and vitamin D malabsorption (Danielsson et al 1982). In the present study one patient had osteomalacia and also symptoms of oligoarthritis.

Lung function abnormalities (IV)

In 25 PBC patients, 14 (56%) had abnormal lung function tests. However, only one patient out of 7 with asymptomatic liver disease had a lung function that deviated from the predicted. None of the reference subjects presented an obstructive or restrictive spirometric pattern and all had DLCO above 70% of predicted value. Four patients had chronic obstructive lung disease; one had airway obstruction with a severe lung

emphysema (a non-smoker with normal α-1-antitrypsin) appearing as hyperinflation on a chest radiogram. Chest X-ray was otherwise normal in all reference and PBC patients. Bronchial asthma was found in 3 patients and in 2 cases the PBC diagnosis preceded the lung symptoms by several years; the third patient had had bronchial asthma since childhood. The comparatively high prevalence of bronchial asthma (12%) could be a coincidence, arising from a small study population. However, the possibility cannot be excluded that one of the extrahepatic manifestations of PBC might be chronic obstructive lung disease. In our patients there was no association between Sjögren's syndrome and an obstructive spirometry pattern as has been suggested by others (Rodriguez-Roisin et al 1981; Segal et al 1981).

A decreased diffusion capacity (DLCO <70% of predicted value) was found in 9 out of 24 (38%) PBC patients. In 18 patients in whom DLCO as well as working capacity were obtained, a significant correlation was found between these parameters (r=0.74). The mean age of the patients with decreased DLCO was 50 years compared to 56 years for those with normal DLCO. Decreased DLCO could be an effect of smoking (Van Ganse 1972), but smoking was uncommon in both groups studied and does not explain the results. There was no overrepresentation of KCS among those with impaired diffusing capacity, in contrast to earlier reports (Rodriguez-Roisin et al 1981). In PBC lung function tests have demonstrated either impairment of a restrictive (Golding et al 1973), or an obstructive pattern (Clarke et al 1978), as well as decreased DLCO (Rodriguez-Roisin et al 1981). All these findings have been attributed to the concomitant presence of connective tissue diseases known to have a high prevalence in PBC. However, the present study does not lend support to such an association.

Sjögren's syndrome (V)

The term "sicca complex" or "sicca syndrome" has often been used to describe the combination of KCS and xerostomia (XS), but has lately been replaced by the term primary Sjögren's syndrome (PSS) (Frost-Larsen et al 1978). The definition of secondary SS is the combination of KCS and/or XS in combination with various autoimmune diseases such as rheumatoid arthritis, SLE, scleroderma or PBC (Moutsopoulos 1980). The association between PBC and SS is well documented in the literature (Alarcón-Segovia et al 1973; Crowe et al 1980a; Culp et al 1982; Giovannini et al 1985). SS is, by definition, directly connected with rheumatic disorders, but an association between SS and pulmonary dysfunction has also been described. Moreover, a wide range of pulmonary abnormalities have been reported in patients with SS (Shearn 1977). The question has been raised as to whether PBC is connected with rheumatic disorders and pulmonary abnormalities per se or whether it constitutes a part of SS.

In the present investigation (V) one or more features of SS were found in 73% of the patients; 54% (14) had positive staining with rose bengal, 35% (9) had Schirmer test of less than 5 mm and in 25% (6/24) the sialograms showed sialectasia. In the literature the frequency of SS in PBC varies from 26 to 72 % (Golding 1973; Hamlyn 1980; Tsianos et al 1990). Other liver diseases, such as chronic active hepatitis are also known to have an association with KCS and/or xerostomia, but not as commonly as in PBC (Golding 1973). The interpretation of different studies is difficult, because of the lack of universal agreement on the criteria for SS (Manthorpe & Prause 1986).

To fulfil the criteria for SS not only was corneal staining by rose bengal was obligatory, but also a Schirmer test of less than 5 mm in 5 min and/or sialectasia demonstrated on sialography. According to these criteria 27% (7) had SS. Using only a rose bengal with positive corneal

staining as criteria for KCS, Culp et al (1982) found that 66% had SS in a material of 113 PBC patients. If the same criteria were applied to the present material 46% would have been classified as SS. Crowe et al (1980a) used the definition of a Schirmer test of less than 5 mm in a material of 95 PBC patients and found that 37% were abnormal, which is consistent with the present results. Rose bengal is sensitive to demonstrated epithelial damage, but the specificity is low. Therefore, in our opinion rose bengal should always be combined with a Schirmer test in diagnosing KCS. The Schirmer test alone is unreliable (Wright & Meger 1962).

There was a good correlation between KCS and the presence of sialectasia - only one patient of the 6 presenting sialectasia did not fulfill the criteria for KCS. Sialectasia in SS varies from 50 to almost 100 % (Bloch 1965; Whaley 1972). The cause of sialectasia is not only autoimmune, but could also be a result of various infections and is rarely congenital (Ericson 1973). None of the patients had any history of infection in the parotid region. Apart from the sialectasia, there was a higher prevalence of segmentation and atrophy than could be expected in a normal material (Ericson 1973). In general these abnormalities were combined with the finding of sialectasia. However, gracile ducts were present in four patients and although it was regarded as normal, two of them had xerostomia.

In 7 PBC patients with SS, 3 displayed decreased diffusion capacity and/or arthritis (Fig. 3). Therefore, arthritis and/or decreased diffusion capacity seem to occur alone and not only in combination with SS. Only 2 out of the 7 asymptomatic PBC had SS and none had arthritis or decreased diffusion capacity. This may indicate that symptomatic PBC is a more widespread form of the disease. The impairment of Schirmer or rose bengal tests did not correlate with disease duration and/or histological stage of the liver disease, which is in contrast to a previous

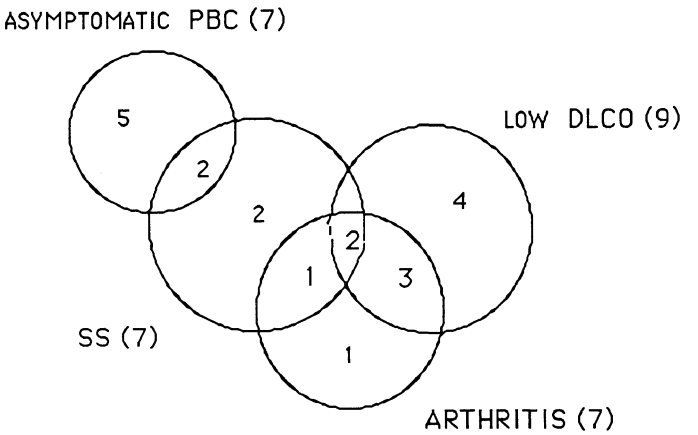

Fig. 3. Correlation between Sjögren's syndrome and arthritis, low diffusion capacity (DLCO) and asymptomatic PBC in 26 consecutive patients.

study (Giovannini et al 1985). Earlier investigations have found a relation between SS and HLA-B8 (Fye 1976), but later in differentiating between primary and secondary SS the association with HLA-B8 disappeared when KCS and/or xerostomia was found in combination with rheumatoid arthritis or PBC (Hamlyn 1980; Moutsopoulos 1980). None of the PBC patients with SS were HLA-B8 positive, whereas 27% of all PBC patients were HLA-B8 positive, which is about the frequency expected in the background population thus confirming the lack of association between HLA-B8 and secondary SS.

PROGNOSIS AND PROGNOSTIC MARKERS (VI)

PBC is a chronic liver disease often with a progressive course, but the prognosis may vary considerably among individual patients. The five-year survival rates vary also between different studies (Sherlock & Scheuer 1973; Roll et al 1983; Christensen et al 1985). Asymptomatic patients may have a normal life expectancy when matched for age and

sex (Beswick et al 1985). Death rates from PBC are difficult to establish, but are probably at the most 3.2% of patients dying from liver cirrhosis (Hamlyn & Sherlock 1974). The methods for evaluating the prognosis of the individual patient with PBC are inefficient. Liver transplantation is the ultimate treatment of end-stage PBC and there is a need for parameters for predicting the outcome of the disease and for establishing the optimal time for transplantation.

Of 111 patients, 25 died during the study period and 14 died as a direct consequence of the liver disease; 10 of hepatic coma and 4 of upper gastrointestinal bleeding. These patients had had signs of liver disease for a mean of 10 years (range 1-22). Only 21% of the patients who died from PBC were asymptomatic at the start of the study period, as compared to 73% of all PBC patients. Early studies of PBC have found a short survival time after the onset of symptoms (Ahrens et al 1950; Sherlock 1959; Sherlock & Scheuer 1973). Later, in a large study Roll et al (1983) showed that the mean survival time was 11.9 years, twice that reported earlier. In previous as well as the present study from Sweden, the good prognosis for asymptomatic patients without hepatomegaly has been confirmed (**I**; Nyberg et al 1988; 1989).

Greater age, hepatomegaly, jaundice, cirrhosis, and signs of hepatic decompensation correlate with a poor prognosis (Shapiro et al 1979; Christensen et al 1985). Three patients (12%) developed hepatocellular carcinoma, one being asymptomatic without any evidence of cirrhosis. Hemochromatosis and chronic active hepatitis with cirrhosis are considered to have a high risk of developing hepatocellular carcinoma, 36% and 42% respectively, whereas PBC has been reported to carry a low risk with an incidence of only 3% (Johnson et al 1978; Krasner et al 1979). Hepatocellular carcinoma has been described earlier in one precirrhotic patient with PBC (Gluskin et al 1985).

A low serum albumin level and/or prolonged prothrombin time, which is not corrected by parenteral vitamin K administration indicate advanced liver disease and a poor prognosis, as does an elevated serum bilirubin level (Shapiro et al 1979). Hyaluronan is a high molecular weight polysacharide which is synthesized largely by mesenchymal cells and degraded by the endothelial cells of the liver. Nyberg et al (1988) have shown that plasma hyaluronan is increased in the advanced histological stage of the disease. Moreover the level of hyaluronan correlates with albumin, galactose tolerance test, serum bilirubin and prothrombin time. Another serum marker is N-terminal propeptide of collagen type III (P III P) which it has been claimed can identify patients with a poor prognosis (Eriksson & Zettervall 1982).

Table III: Clotting factors in PBC and hepatocellular liver diseases

	C1Inh (μmol/l)	AT (%)	vWF (%)	PC (%)
Reference values	1.3-1.9	80-120	60-160	70-130
Controls (n=22)	1.53±0.049	ND	113±9	ND
Hepatocellular disease (n=11)	1.36±0.14^{ns}	41±5	347±39***	48±4
PBC (n=27)	1.95±0.091***	94±6	199±18***	85±6
Asymptomatic (n=14)	1.84±0.12*	102±5	147±10*	99±5
Symptomatic (n=13)	2.06±0.14**	83±13	267±29***	68±11

Mean values ± SEM, * $p<0.05$, **$p<0.01$ and *** $p<0.001$ for comparison with controls (n=22).
ND = not done, C1Inh = P-C1-inhibitor, AT = P-antithrombin, vWF = P-von Willebrand factor, PC = Prothrombin complex

Plasma concentrations of C1 inhibitor, antithrombin, vWF and prothrombin complex were studied in groups of asymptomatic and symptomatic PBC patients and the results were compared with those obtained for hepatocellular disease (Table III). In patients with hepatocellular liver disease plasma antithrombin and prothrombin complex were reduced, whereas they remained fairly normal in PBC. On the other hand, the vWF was markedly elevated in both groups. C1 inhibitor was normal in hepatocellular disease but increased in PBC patients suggesting an induction of synthesis by cholestasis. Symptomatic PBC differed from asymptomatic PBC in having more marked increases in vWF and C1 inhibitor levels, although the latter difference was not statistically significant. The present investigation indicates that the vWF could be used as an indicator of hepatocellular function in PBC.

In cholestatic liver diseases such as PBC the synthesis of liver-produced coagulation proteins seems to be increased (Cederblad et al 1976). Antithrombin concentrations appear to decrease in parallel with a reduction in the functioning liver parenchyma (Rodzynek et al 1984), in contrast to the vWF, which may reach very high values in fulminant liver failure (Maisonneuve & Sultan 1977; Langley et al 1985). Patients with PBC and a well-preserved function of the liver display no gross changes in antithrombin in contrast to those with alcoholic cirrhosis (Sinclair et al 1988).

GENERAL SUMMARY AND CONCLUSIONS

1. During 1973-1982 the mean annual incidence of PBC was 13.3 per 10^6 and the point prevalence for 1982 was 151 per 10^6 inhabitants in the Northern Health Region of Sweden (570,000 inhabitants). The incidence rate is similar to earlier swedish findings, but higher than for the rest of Europe, whereas the prevalence seems to be the highest reported so far.

2. The finding of a negative Kveim test in combination with a positive AMA (titre 1/25 or more) seems to be of great value in differentiating between PBC and sarcoidosis.

3. Rheumatic disorders were found in about 50% of the patients and 27% had arthritis. Rheumatic disorders are common in PBC patients, whether or not they are symptomatic, and sometimes joints symptoms may even dominate the clinical picture.

4. Lung function abnormalities were found in 56% of investigated patients and almost exclusively in symptomatic PBC, perhaps indicating a more widespread form of the disease. A spirometric pattern of the obstructive type and decreased DLCO (36%) were the most common findings of pulmonary engagement.

5. Features of Sjögren's syndrome were found in 73%, but only 7 patients fulfilled the criteria for SS. Only 3 had arthritis and/or decreased diffusion capacity whereas 8 had not, indicating that joint- and pulmonary symptoms in PBC are not mediated by SS.

6. Determination of C1 inhibitor levels cannot be used as a measurement of residual hepatocyte function. However, the present study suggests that determination of the von Willebrand factor may be more suitable for this purpose.

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